Nature of Mechanoradical Formation of Substituted Celluloses as Studied by Electron Spin Resonance

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Mechanically induced free radical (mechanoradical) formation of several substituted celluloses such as carboxylmethyl cellulose, chitin, and chitosan was studied based on electron-spin resonance (ESR) in comparison with those of plasma-induced radicals. Room temperature ESR spectra had multicomponent spectra and were different in pattern from each other. The mechanoradical concentration gradually decreased after reaching the maximum value in each substituted polysaccharide, accompanied by a decrease in molecular weight in the course of vibratory milling. One of the most intriguing facts is that the component radicals are all glucose-based radicals as in the case of plasma irradiation, although it is known that mechanoradicals are formed by 1,4-glucosidic bond cleavage of polysaccharides.

Key words mechanoradical; polysaccharide; electron spin resonance (ESR); computer simulation

Over the years, we have reported on the electron-spin resonance (ESR) elucidation of the plasma-induced radicals of a variety of glucose-based saccharides, such as *myo*-inositol,[1] monosaccharides (α- and β-glucose),[2] disaccharides (maltose and cellobiose),[3] and polysaccharides (cellulose and amylose),[4] ethyl- and hydroxyethylcellulose (EC and HEC),[5] and low- and high-substituted hydroxypropylcellulose (L-HPC and H-HPC).[6] We have further undertaken the characterization of the plasma-induced radicals of several other substituted celluloses such as carboxylmethylcellulose (CMC), 2-acetamido-2-deoxycellulose (chitin), and 2-amino-2-deoxycellulose (chitosan)[7] in response to the need for experimental design in a series of studies on novel plasma-assisted drug-delivery system (DDS) preparations.[8—19] The substituted celluloses thus far studied are all pharmaceutically important polymers used as pharmaceutical aids and food additives. The study of mechanolysis is of fundamental significance in connection with the manufacture of a wide variety of solid materials in industry. Polymers including polysaccharides in powdered form are also often used in a variety of industrial fields. It has been known that mechanolysis of polymers, synthetic and natural, results in mechanically induced radicals, so-called mechanoradicals, due to the polymer main-chain scission when the glass transition temperature (T_g) of the polymers exceeds the temperature at which the mechanolysis is conducted. Little attention has been paid, however, to the formation of mechanoradicals in many fields of industry including pharmaceutical engineering, although mechanoradical formation results in more or less the same changes in physicochemical properties resulting from the decrease in molecular weight due to main-chain scission and its ensuing processes such as the reaction of recombination and disproportionation.

ESR studies of mechanoradical formation reported previously dealt with polymers ground at low temperature (77 K),[20] and no such study at room temperature has been carried out, although in practice most operations of grinding and/or ball milling are conducted at room temperature.

We reported detailed analyses of room-temperature ESR spectra of the mechanoradicals formed in synthetic polymers[21,22] and polysaccharides such as cellulose and amylose[23] under strictly anaerobic conditions, and discussed the novel and significant features of mechanoradical formation and changes in physicochemical properties caused by the mechanolysis.

In this paper, we report on mechanoradical formation and the reactivities of several pharmaceutically important substituted celluloses such as CMC, chitin, and chitosan (Fig. 1), in comparison with those of plasma-irradiated samples, as studied by ESR coupled with systematic computer simulations. Among various substituted celluloses, we selected in the present study three derivatives with substituents capable of intense intermolecular interactions.

Experimental

Materials Powdered CMC (degree of substitution: 0.4—0.6[24,25]) was kindly supplied by Gotoku Chemical Co., Ltd. (Japan). Powdered chitin and chitosan (degree of deacetylation: 0.8—1.0) were kindly supplied by Kimitsu Chemical Industries Co., Ltd. (Japan). They were screened with a 200—235 mesh sieve and dried at 70 °C for 12 h *in vacuo*.

Method of Mechanolysis Powdered samples (150 mg) were mechanically fractured at 60-Hz frequency using vibratory ball milling [stainless steel ball (6.0 mmϕ, 890 mg) and a stainless steel twin-shell blender (7.8 mmϕ, 24 mm long), Shimadzu Co., Ltd.] at room temperature for the prescribed period of time. The fractured samples were transferred to an ESR tube, which was then sealed and submitted to ESR measurement. All these operations were carried out in a vacuum glove box (Sanplatec Corp., Japan). Air in the vacuum glove box was replaced with purified nitrogen gas and then the remaining oxygen in this system was removed with a High Capacity Gas Purifier (Supelco, Inc., Japan). The oxygen concentration was monitored with an oxygen analyzer (LC 750/PC/120, Toray Engineering Co., Ltd.) and kept below 0.01 ppm.

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 microwave power level of 0.01 mW was chosen. The ESR spectral intensity was determined using double integration. The radical concentrations (spin numbers/g) were calculated from the spectral intensities with the aid of calibrated lines obtained from the spectral intensities of a polymethylmethacrylate (PMMA) sample impregnated with DPPH. Measurements of *g*-values were made relative to the fourth signal from the lower magnetic field (g=1.981) of Mn^{2+} in MgO.

Computer Simulation of ESR Spectra Computer simulations were performed on a 32-bit microcomputer (NEC PC9801FA). The simulated spectra were obtained from Lorentzian functions by fitting iteratively the spectroscopic parameters [*g*-value, line width at half-height, hyperfine split-

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ting constant (HSC), and relative intensity] iteratively with the observed digitized spectra using the nonlinear least-squares method.[4] The simulation programs were designed 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[26] and Cochran's equation,[27] respectively.

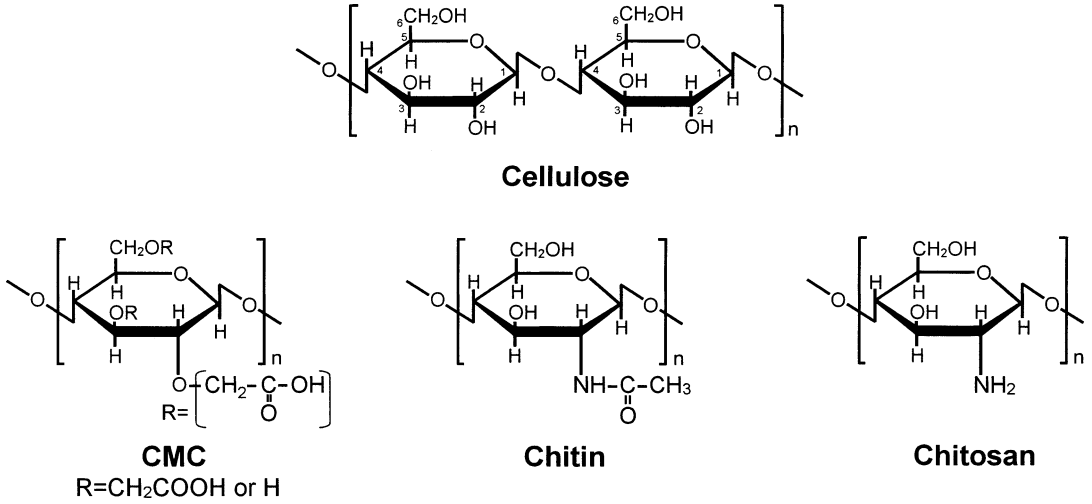

Fig. 1. Structures of CMC, Chitin, and Chitosan

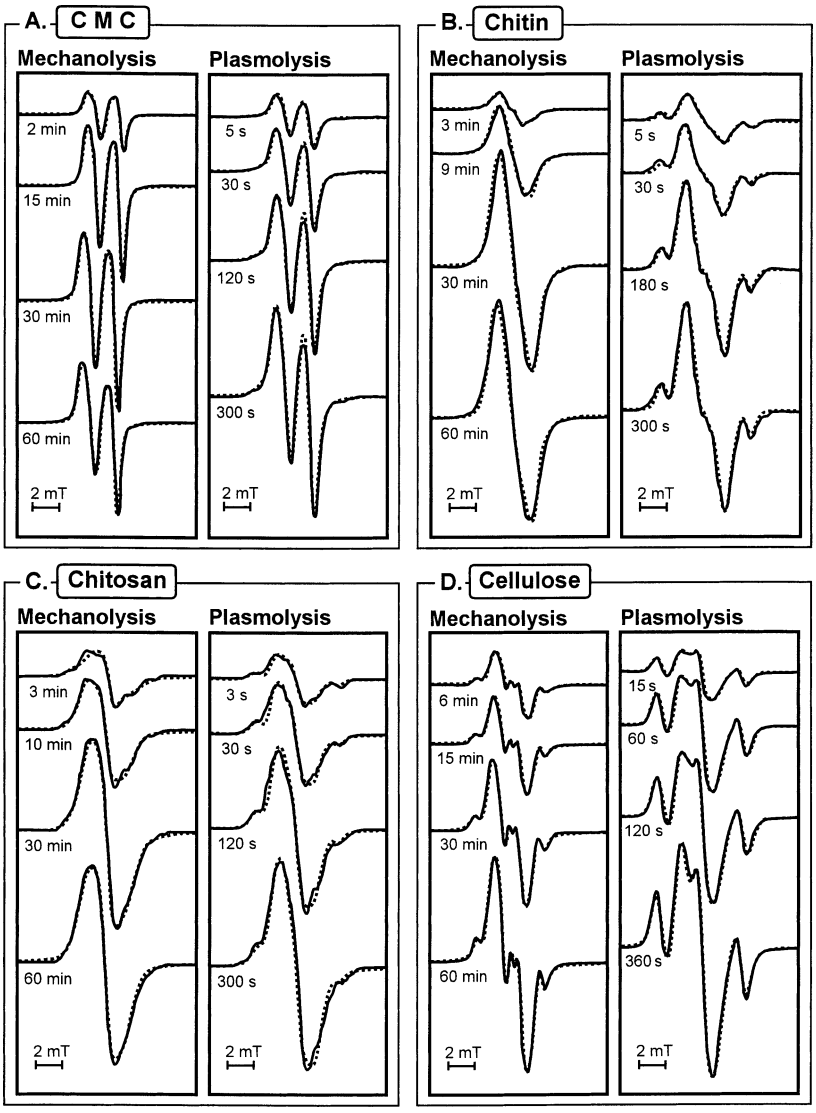

Fig. 2. Observed Time-Course ESR Spectra of CMC (A), Chitin (B), Chitosan (C), and Cellulose (D) Radicals Formed by Mechanolysis and Plasmolysis Together with the Simulated Spectra Shown as Dotted Lines

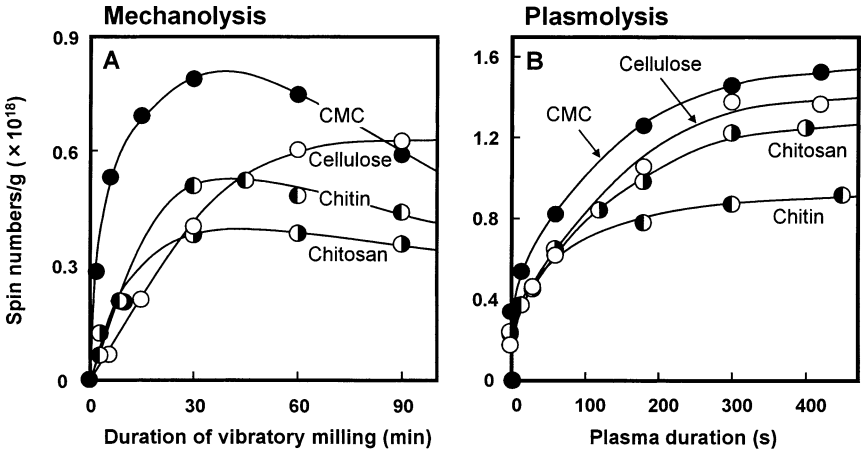

Fig. 3. Progressive Changes in Spectral Intensities Determined by Double Integration in the Course of Vibratory Milling (A) and on Plasma Duration (B)

Results and Discussion

Observed ESR Spectra of Substituted Polysaccharide Radicals Formed by Mechanolysis Figure 2 shows the progressive changes in the observed ESR spectra of CMC, chitin, chitosan, and cellulose radicals formed by mechanolysis, together with those of cellulose by plasmolysis for comparison.

It is seen from Fig. 2 that the spectral features vary with the nature of substituents in a sensitive manner, but remain nearly unchanged in the course of mechanolysis as well as plasmolysis in each case. These spectra appear to be an outline of multicomponent spectra and also apparently differ from those of cellulose shown in Fig. 2D.

Comparing the observed ESR spectra of CMC and chitosan between mechanolysis and plasmolysis, the spectral features are similar to each other, while the observed spectra in the mechanolysis of chitin and cellulose were different from those of plasmolysis, respectively, in terms of the lack of the lateral peaks on the outer side of the higher and lower field of the major central peaks in the case of mechanolysis.

Figure 3 shows the progressive changes in total spectral intensities in the course of vibratory milling (Fig. 3A) and plasma duration (Fig. 3B), which were determined through double integration of the observed spectra.

It can be seen from Fig. 3A that the radical concentration more readily decreased after reaching the maximum value in each polysaccharide, although cellulose required much longer vibratory milling (120 min) to exhibit the maximum value in the mechanoradical concentrations.[23] In the case of plasmolysis, however, the radical concentrations tended to level off in all plasma-irradiated polysaccharides (Fig. 3B).

We have experimentally demonstrated in the mechanolysis of cellulose that the molecular weight decrease toward the limiting value due to polymer mainchain scissions, *i.e.*, the cleavage of 1,4-glucosidic bonds, and the rate of the mechanoradical formation decreases accompanied by a decrease in the molecular weight.[23] Consequently, the time-course changes in mechanoradical concentration shown in Fig. 3A indicate that the ensuing reactions of the mechanoradical formed exceed the radical generation, as the molecular weights of polysaccharides come close to the limiting value, and that substituted celluloses are more prone than cellulose to undergo the ensuing radical reactions such as a recombination reaction due to mechanical force.

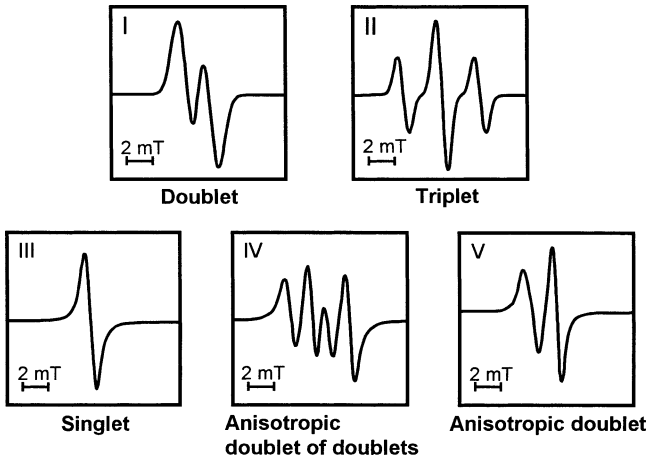

Fig. 4. Component Spectra (I—V) for the Simulated ESR Spectra

Corresponding Simulated Spectra We have systematically conducted computer simulations of these progressive changes in the complicated spectra in an interrelated manner. The simulated spectra corresponding to the observed spectra are shown in Fig. 2 by dotted lines. It can be seen that all the observed spectra were satisfactorily reproduced by the simulated spectra.

Figure 4 shows the representative spectral components of the simulated spectra. The computer simulations showed that the spectra of chitin and chitosan both in mechanolysis and plasmolysis consisted of three types of discrete spectral components, two isotropic spectra [doublet (I) and triplet (II)], and one anisotropic spectrum [doublet of doublets (IV)], and a single broad-line spectrum (III). These component spectra ((I)—(IV)) are essentially identical in nature to those of cellulose.[23] It should be noted that the spectra of CMC contain a considerable amount of an anisotropic doublet (V), in addition to (I), (III), and (IV). All simulated spectra were obtained from admixtures of the component spectra by varying the ratios. Thus our systematic simulations of progressive spectral changes eliminate any fortuitous line coincidence in obtaining the simulated spectra and ensures the reliability of the results of our simulations.

The ESR spectroscopic parameters for a representative se-

lection of these component spectra deduced from the systematic simulations are summarized in Table 1. The principal anisotropic parameters vary somewhat with the spectra so that their values are only of semiquantitative significance.

Progressive Changes in Component Spectra Figure 5 shows the progressive changes in the spectral intensity of

Table 1. ESR Spectral Data for Component Radicals in Simulated Spectra of Plasma-Irradiated CMC, Chitin, and Chitosan[a)]

		I	II	III	IV	V
CMC						
	g	2.0035		2.0041	$\bar{g}=2.0037$ $\left\{\begin{array}{l} g_1=2.0032 \\ g_2=2.0033 \\ g_3=2.0048 \end{array}\right.$	$\bar{g}=2.0031$ $\left\{\begin{array}{l} g_1=2.0017 \\ g_2=2.0033 \\ g_3=2.0044 \end{array}\right.$
	A_α				$\bar{A}_\alpha=1.63$ $\left\{\begin{array}{l} A_1=1.40 \\ A_2=1.55 \\ A_3=1.94 \end{array}\right.$	$\bar{A}_\alpha=1.88$ $\left\{\begin{array}{l} A_1=1.29 \\ A_2=1.47 \\ A_3=2.87 \end{array}\right.$
	A_β	1.70			$\bar{A}_\beta=2.94$ $\left\{\begin{array}{l} A_1=2.94 \\ A_2=2.94 \\ A_3=2.94 \end{array}\right.$	
Chitin						
	g	2.0034	2.0027	2.0040	$\bar{g}=2.0029$ $\left\{\begin{array}{l} g_1=2.0023 \\ g_2=2.0025 \\ g_3=2.0039 \end{array}\right.$	
	A_α				$\bar{A}_\alpha=1.63$ $\left\{\begin{array}{l} A_1=1.40 \\ A_2=1.55 \\ A_3=1.95 \end{array}\right.$	
	$A_\beta(1)$	1.70	2.98		$\bar{A}_\beta=2.94$ $\left\{\begin{array}{l} A_1=2.94 \\ A_2=2.94 \\ A_3=2.94 \end{array}\right.$	
	$A_\beta(2)$		2.98			
Chitosan						
	g	2.0042	2.0036	2.0044	$\bar{g}=2.0045$ $\left\{\begin{array}{l} g_1=2.0040 \\ g_2=2.0040 \\ g_3=2.0054 \end{array}\right.$	
	A_α				$\bar{A}_\alpha=1.63$ $\left\{\begin{array}{l} A_1=1.40 \\ A_2=1.55 \\ A_3=1.95 \end{array}\right.$	
	$A_\beta(1)$	1.70	2.78		$\bar{A}_\beta=2.94$ $\left\{\begin{array}{l} A_1=2.94 \\ A_2=2.94 \\ A_3=2.94 \end{array}\right.$	
	$A_\beta(2)$		2.78			

a) Values of HSC are given in mT.

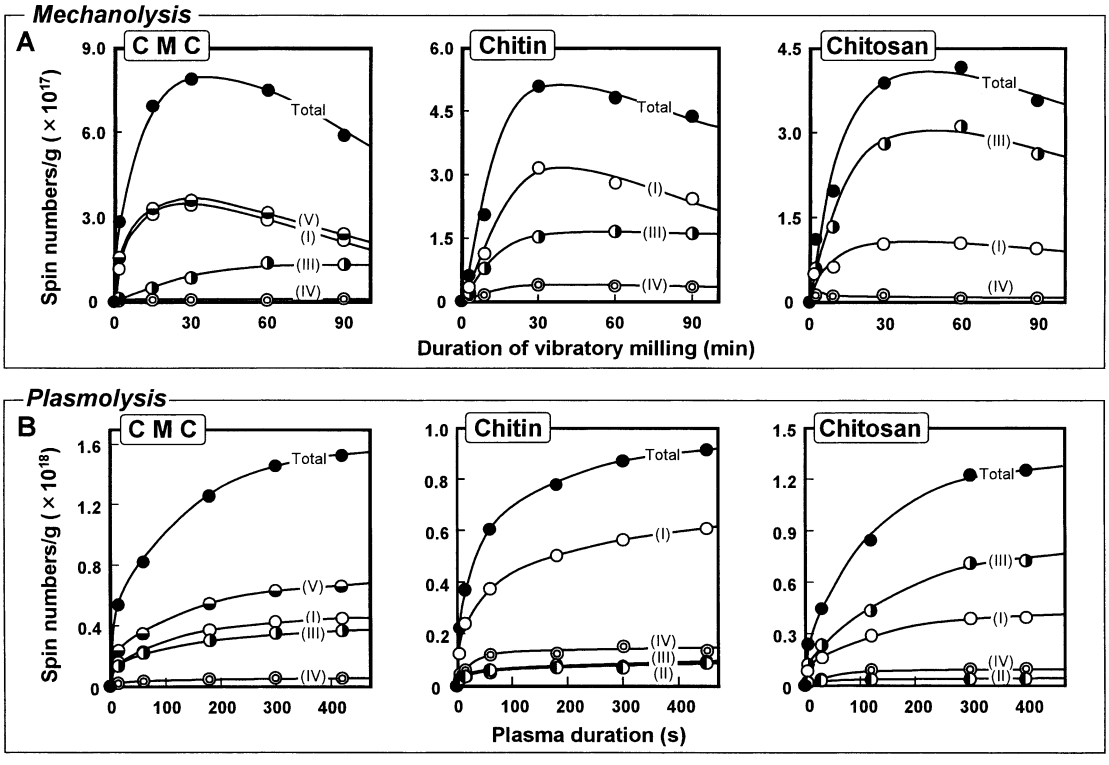

Fig. 5. Progressive Changes in Each Component Spectral Intensity Corresponding to the Simulated Spectra on Mechanolysis (A) and Plasmolysis (B)

each component radical corresponding to the simulated spectra shown in Fig. 2. The total spectral intensities were very compatible with the intensities determined through double integration of the observed spectra. It is apparent from Fig. 5 that the ratio of change in each spectral intensity does not vary much with component spectra, accounting for rather small spectral changes in pattern in the course of mechanolysis and plasmolysis in each case. Although the broad single-line spectrum was the major spectral component in the mechanolysis of both cellulose and amylose,[23] this was not the case for substituted celluloses.

The observed ESR spectra of the radicals formed in chitin and chitosan by mechanolysis were appreciably different in pattern from those of plasmolysis, as in the case of cellulose. This anomaly can be understood in terms of the fact that the triplet (II) detected in plasmolysis scarcely formed in mechanolysis.

Structural Assignments of Mechanoradicals and the Reaction Sequence Although it is known that the mechanoradicals are formed by polymer main-chain scission, *i.e.*, 1,4-glucosidic bond cleavage for glucose-based polymers,[28] the room-temperature mechanoradicals observed are all glucose-derived midchain alkyl radicals similar to those in plasmolysis. Such end-chain radicals, however, could not be detected during room-temperature mechanolysis based on our systematic computer simulations. As shown in Fig. 6, the 1,4-glucosidic bond scission of polysaccharide can formally give two pairs of end-chain radicals; two types of alkyl radicals, X and X′; and two alkoxy radicals, Y and Y′. Such end-chain radicals may have rigorous molecular motion and high reactivities. Thus such radicals could abstract a hydrogen atom of the surrounding glucose units to give hydroxyalkyl radicals (1) and/or could undergo radical–radical coupling to give nonradical species, accounting for the formation of the glucose-derived mid-chain alkyl radicals similar to those on plasma irradiation. It may be difficult otherwise to interpret the observed experimental results.

A nearly isotropic doublet with *ca.* 1.7 mT of HSC (I) can

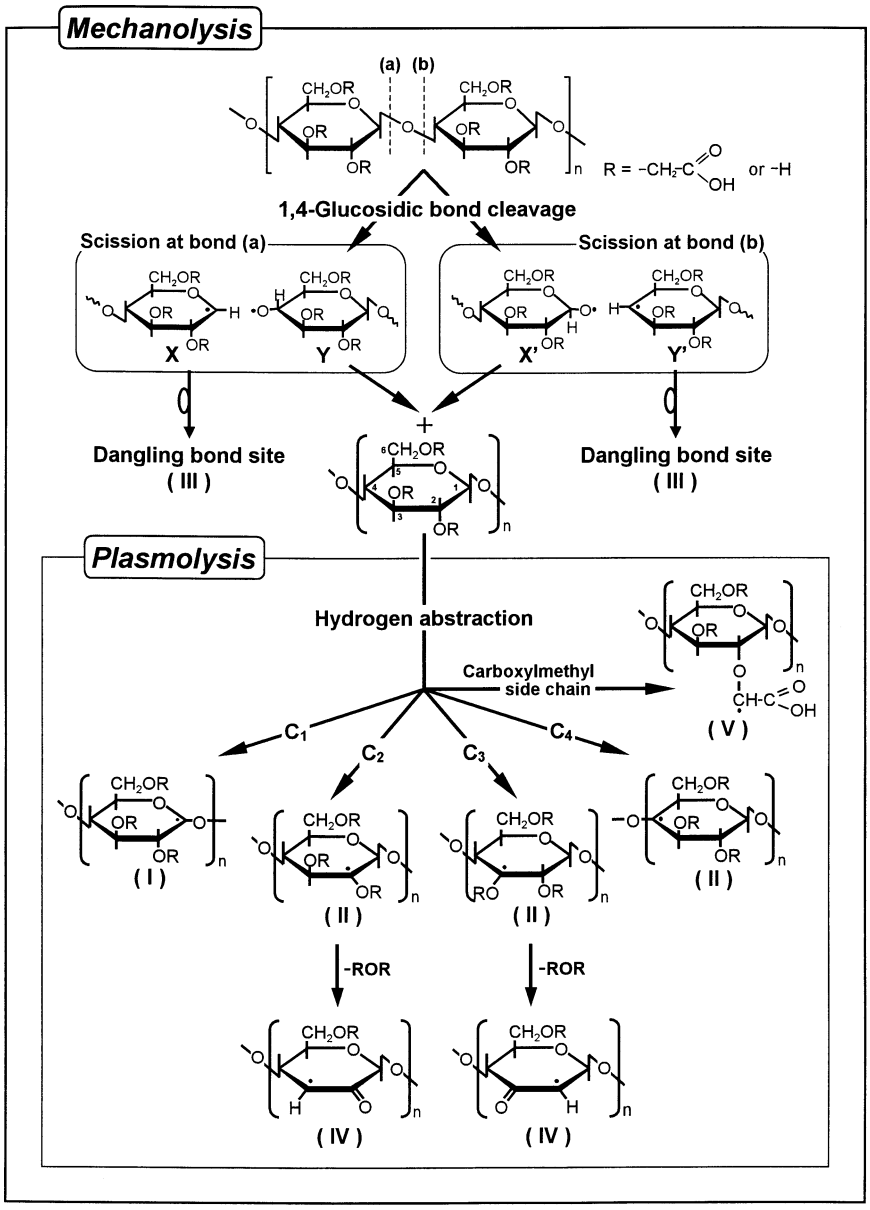

Fig. 6. Structures of Mechanically Induced Radicals in Substituted Polysaccharide and the Reaction Sequence

be assigned to an alkoxylalkyl radical (1) formed by a hydrogen abstraction at C1. Based on the cosine square rule, the rather large *g*-values and smaller HSC of the doublet for axial β-hydrogen at C2 may stem from the influence of the two oxygens bonded to the radical center. The isotropic triplet (II) in chitin and chitosan is most reasonably assigned to an aminoalkyl radical at C2, a hydroxylalkyl radical at C3, and/or an alkoxylalkyl radical at C4 split by two axial β-hydrogens. However, the present ESR spectra cannot discriminate these alternatives. Further, we can assign a doublet of doublets (IV) with *g*-factor and hyperfine anisotropy in CMC to an acylalkyl radical at C2 (6) and/or C3 (5), which resulted from the facile dehydration and/or dealkoxylation of a hydroxyl and/or alkoxylalkyl radical at C3 (3) and/or C2 (2). Similarly, in chitin and chitosan we assume that the similar acylalkyl radical at C2 is formed by the facile deamination due to the presence of an α-hydroxylalkyl radical at C3.

One of the noteworthy features is that the ESR spectra of plasma-irradiated CMC contain a considerable amount of anisotropic doublet (V), which is a major component spectrum in CMC. We can assign the anisotropic doublet (V) to an acylalkyl radical (7) formed at the carboxylmethyl side chain of CMC, as shown in Fig. 6. The radicals formed at substituents in cellulose can generally be considered to be unstable, since they have a high degree of freedom even in the solid state. In the case of CMC, however, the acylalkyl radical (7) formed at the carboxylmethyl side chain can be stabilized due to the capability of strong intersegmental hydrogen-bonding networks associated with carboxylic groups. The anisotropies of (V) are caused by *g*-factor from conjugated carbonyl groups and HSC from α-hydrogen.

Finally, although the *g*-value of the single broad line (III) is a carbon-centered, not oxygen-centered, radical, we cannot present any discrete radical structure for (III). This radical may be a mixture of ring-opened and/or conjugated structures resulting from 1,4-glucosidic bond cleavages followed by its ensuing complex reactions and of no structural significance.

Conclusion

We have presented the detailed analysis of radicals formed by mechanolysis of CMC, chitin, and chitosan in comparison with those formed by plasmolysis based on the ESR spectra coupled with systematic computer simulations. From the present study, the radicals formed in CMC, chitin, and chitosan were elucidated. The spectra appear to be an outline of multicomponent spectra and also apparently differ from those of cellulose. Noteworthy features are that the spectral features vary with the nature of substituents in a sensitive manner and that the radical concentration decreased readily after reaching the maximum value in each substituted polysaccharide, unlike the case of cellulose.

It was also found that the observed mechanoradicals are all glucose-based midchain alkyl radicals similar to those after plasma irradiation, although it is known that the mechanoradicals are formed by 1,4-glucosidic bond cleavage of polysaccharides. It can be reasonably assumed, therefore, that the mechanoradicals primarily formed underwent hydrogen abstraction from the glucose unit to result in glucose-based radicals even in mechanolysis. Interestingly, the observed ESR spectra of CMC radicals contained a considerable amount of anisotropic doublet (V) and were assigned to the acylalkyl radical formed at the carboxylmethyl side chain of CMC. The stability of (V) was ascribed to the capability of intersegmental hydrogen-bonding networks associated with carboxylic groups in CMC.

The present findings also provide a basis for the future methodological criteria to prepare the desired and functionalized composite powders using polysaccharides. It is noteworthy that, although we used rather stringent conditions for anaerobic mechanolysis in the present study, our results suggest that one should be more cautious in the grinding and milling of pharmaceutical additives, even under aerobic conditions.

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