Motion of Spin Labels Covalently Attached to Cellulose

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High molecular weight (DP $\gg$ 1000) cellulose has been tosylated and subsequently substituted with 2,2,6,6-tetramethyl-4-aminomethyl-piperidin-1-oxyl. The dynamic properties of the spin-labeled cellulose were analyzed by simulation of the line shape of its ESR spectra in the slow tumbling region. The over-all motion of the nitroxyls was found to be aniso-tropic with a ratio of four between the axial and the non-axial components of the motion, and the correlation time is 1.6×10^{-8} s rad^{-1} at 40 °C.

Paramagnetically covalently and non-covalently labeled systems have been used to study motion, environment and conformation in biological macromolecules and in synthetic polymers. Excellent reviews on these subjects have been published.[1] Despite the rapid development in this field very few investigations on the spin-labeling of polysac-charides are to be found in the literature. Gagnaire and Odier[2] report the selective covalent nitroxyl-labeling of cellulose acetate of moderate molecular weight, degree of polymerization, DP $\simeq$ 50. Cafe et al.[3] attached nitroxyl groups to sodium carboxy-methyl cellulose (DP $\sim$ 200). Darcy and Mc Geeney[4] used spin-labeled amylose (DP $\sim$ 90) to study enzymatic depolymerization. The aim of the present work is to produce a high molecular weight nitroxyl-labeled cellulose and to study the characteristics of the over-all motion of nitroxyl groups with respect to the cellulose molecules by comparison of experi-mental and simulated ESR spectra.

EXPERIMENTAL

Synthesis of spin-labeled cellulose. Cotton linter pulp SR-5E of Buckey type 1 A 500-2 was used as starting material. The DP of the sample was $\gg$ 1000,

approximately 1900 as determined in cadoxen.[5] Tosylation (introduction of *p*-toluene sulfonyl groups) was accomplished by the procedure of Malm et al.[6] and yielded a product with a degree of sub-stitution, DS = 0.71, with respect to tosyl groups. 180 mg of swollen cellulose tosylate and 50 mg 2,2,6,6-tetramethyl-4-aminomethyl-piperidin-1-oxyl[7] (*1*) were shaken in 15 ml dry benzene for 21 days at 27 °C. The solid product was thoroughly washed with ethanol and chloroform, and dried. Elementary analysis of the product gave 0.33 % N and 8.08 % S. Nitroxyl *1* was adsorbed to cellulose in a separate experiment. 180 mg swollen cotton linter and 50 mg *1* were shaken in 15 ml benzene for 3 days at 27 °C. The solid product was thoroughly washed with ethanol and chloroform.

IR spectra were measured from potassium bromide pellets on a Perkin-Elmer 237 spectrometer. *ESR spectra* were measured on Varian E-3 and E-12 (X-band) spectrometers. ESR spectra of the spin-labeled cellulose were measured from solid samples, and of samples swollen in various solvents or dis-solved in a 1:1 mixture of *N*-ethylpyridinium chloride and dimethyl sulfoxide (at +80 °C). Flat quartz sample cells were used (30 × 3 × 0.1 mm).

ESR spectra simulation. Computer programs for the simulation of ESR spectra of slowly tumbling nitroxyls according to Freed[13] were used. The com-ponents of the anisotropic hyperfine and *g*-tensors were obtained by simulation of rigid limit spectra according to Lefebvre and Maruani.[8] The programs were tested with spectra of peroxylamino potassium disulfonate in deuterium oxide and in 85 % glycerol — water. All computations were performed at the Computer Centre of the University of Helsinki.

RESULTS AND DISCUSSION

The introduction of *p*-toluene sulfonyl groups (tosyl groups) into cellulose is a well-known pathway

in the preparation of cellulose derivatives.[9] DS values rising to 0.87 for cellulose tosylates are reported. The DS for the tosylated cellulose estimated from IR spectra[10] was 0.71 in this case. The substitution of amines into cellulose tosylates is slow and incomplete. Hess and Ljubitsh[11] treated cellulose tosylate with ammonia and ethylamine, respectively; the nitrogen content of the product was less than 2 %. In a procedure by Haskins[12] DS values for primary amines 0.5 – 1 were obtained. The conversion of the cellulose tosylate to spin-labeled cellulose with *1* yielded a product with DS = 0.04 with respect to nitroxyl groups. The product also contained tosyl groups, DS with respect to tosyl groups is 0.70. The amount of nitroxyl groups in this very high molecular weight cellulose derivative is the same as reported for nitroxyls in sodium carboxymethyl cellulose[3] where the substitution was accomplished in a homogeneous system. DS values for other spin-labeled polysaccharides were not reported.[2,4] The substitution of *1* into tosylated cellulose is non-specific. The nitroxyl groups introduced may be situated in positions 2, 3 or 6, with a slightly higher probability in positions 2 and 6.[12] Substitution to the reducing as well as the non-reducing ends of the macromolecules cannot be excluded.

With a DS = 0.04 the average minimum distance between the paramagnetic centers is about 12 nm provided the nitroxyl groups are moving unhindered with respect to the cellulose chain. The interaction between the nitroxyls is very weak at this distance and could not be detected in spectra of the labeled product.

Table 1. The components of the hyperfine and g-tensors for labeled cellulose from experiments and rigid limit spectra simulation.

A_{xx}	0.70 mT	g_{xx}	2.0089
A_{yy}	0.49 mT	g_{yy}	2.0064
A_{zz}	3.50 mT	g_{zz}	2.0022

The hyperfine and the g-tensors of the nitroxyl group in the labeled cellulose cannot be considered axially symmetric. It has been stated[1] that the success of ESR spectra simulation of slowly tumbling nitroxyls depends critically on the choice of the axial and non-axial sets of parameters for both the hyperfine and the g-tensors. The axial components of the tensors, as well as the approximate arithmetic sum of the non-axial components were obtained from experimental rigid limit spectra of the labeled cellulose.[14] Values for the non-axial components were estimated by simulation of the rigid limit spectra, Fig. 1a. The procedure was the same as has been decribed by Goldman *et al.*[15] The sets of parameters for the nitroxyl fragment in labeled cellulose are collected in Table 1.

These values were used in the simulation of ESR spectra of slowly tumbling nitroxyl groups in labeled cellulose. For a detailed discussion of the background theory and the choice of the motional models seen Refs. 13 and 16. Two motional models
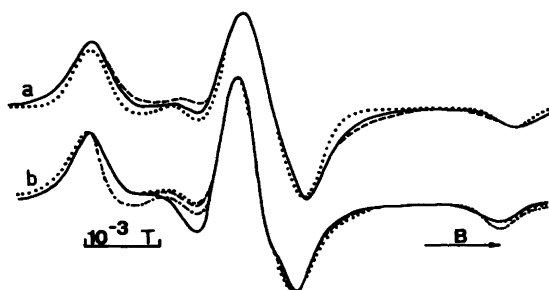
Fig. 1. a. Rigid limit spectra of labeled cellulose: –, Experimental, measured at –100 °C; – – –, Simulated with Lorentzian line-shape; ..., Simulated with Gaussian line-shape. b. Slow tumbling spectra of labeled cellulose: –, Experimental, measured at +40 °C; – – –, Simulated with $N=4$ for free diffusion; ..., Simulated with $N=6$ for free diffusion; .–., Simulated with $N=4$ for Brownian motion. Although the lines are not purely Lorentzian, Lorentzian line-shape gave the best fit in all these cases.
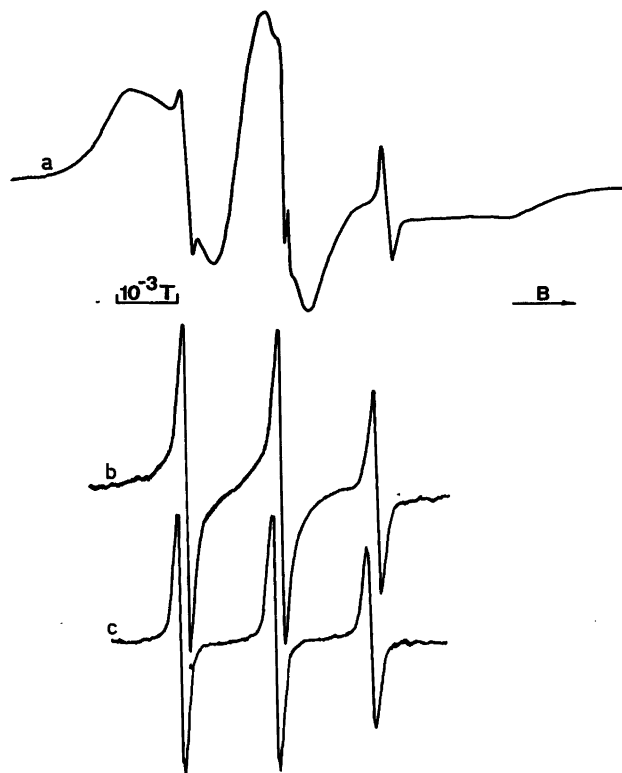
Fig. 2. a. Spectrum of labeled cellulose swollen in water; b. Swollen in dimethyl sulfoxide; c. Dissolved in a 1:1 mixture of N-ethyl pyridinium chloride and dimethyl sulfoxide. a. and b. at 20 °C, c. at 80 °C.

were used; Brownian motion to test if the motion is that of rigidly incorporated nitroxyls and free diffusion as a test for relatively freely reorienting nitroxyls. In contrast to what is assumed for polymeric structures the line shape analysis indicates that the label in dry cellulose obeys free diffusion rather than Brownian motion.[13] The over-all motion of the nitroxyl group is anisotropic, the ratio N between the axial and non-axial components of the motion is four in dry cellulose, the correlation time τ_c is 1.6×10^{-8} s rad^{-1} at $+40$ °C $(\tau_{L,K}^{-1} = (B_L/R_\perp)$ $[R_\perp L(L+1) + (R_\perp - R_\parallel)K^2]$ where B_L is a model parameter, L and K are "quantum numbers" of the Wigner rotation matrices, and $R_\perp$ and $R_\parallel$ the axial and perpendicular components of the rotational diffusion tensor[15]). The nitroxyl group is fixed and exerts only ineffective motion hindered by valence forces and hydrogen bonds between the cellulose chains. Experimental and simulated spectra are given in Fig. 1b.

Labeled cellulose swollen in water shows a two-component spectrum after several days of swelling. This is an indication of the very slow and inhomogeneous swelling of cellulose in water. The rapid motion component arises from nitroxyls bonded in the amorphous regions of crystallites where water penetration is supposed to be fastest. In non-polar solvents (*e.g.* toluene) only a one-component spectrum of very slowly tumbling nitroxyls, similar to the spectrum of dry labeled cellulose, was observed. By swelling in dimethyl sulfoxide rapid motion of the labels is observed immediately after mixing indicated by a purely one-component spectrum with characteristics of anisotropic motion $\left(\dfrac{\Delta B\,(I = +1)}{\Delta B\,(I = 0)} = \dfrac{0.15\ \text{mT}}{0.11\ \text{mT}} \text{ at } 0 \text{ °C},\right.$ indicating preferred rotation around the x-axis[1,15]$\Big)$.

The spectrum of the dissolved labeled cellulose also shows rapid anisotropic motion in a one-component

spectrum. Spectra of swollen and dissolved labeled cellulose are given in Fig. 2.

In a separate experiment spin label *1* was included in cellulose by adsorption from solution. A very small fraction of the label remained adsorbed in the cellulose even after extensive washing. The motion of the adsorbed probe is much faster than for the covalently bonded label, indicated by a one-component spectrum with a total width of 6.25 mT. (6.75 mT in labeled cellulose).[17] The signal of the adsorbed nitroxyl is too weak to be analyzed exactly. The adsorbed component was not observed in the labeled cellulose.

Simulation of ESR spectra of even very high molecular weight compounds labeled with nitroxyl groups gives information on the anisotropy of the over-all motion, the motional model and the rotational correlation time of the nitroxyl group and, therefore, proves to be an efficient method to analyze spectra of nitroxyls in polymer matrices.

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