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Note

Extrinsic Cotton effects exhibited by glucuronans from a dimorphic fungus, *Mucor rouxii**

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Acidic polymers known to adopt a helical conformation in solution have been shown to display optical activity (extrinsic Cotton effects) in the metachromatic band of bound cationic dyes, whereas the non-helical or random coil forms do not[1,2]. This technique of induced circular dichroism (c.d.), using in particular Methylene Blue, has been applied to conformational studies of polysaccharides from animal and algal sources to demonstrate the presence of secondary structures involving net chirality[3,4]. By observations of the c.d. spectra under conditions of different ratios of anionic sites to cationic-dye molecules (P/D), the technique can also be used to investigate the presence of multiple binding sites or variations in polysaccharide structure along the chain[4]. Recently, we have described[5] the isolation of two distinct D-glucuronans (I and II) from both yeast and hyphal walls of the dimorphic fungus *Mucor rouxii*. Polymer I was a heteropolymer containing about 60% of D-glucuronic acid residues, and Polymer II was preponderantly a homopolymer of D-glucuronic acid. Both polymers gave rise to acid-insoluble homopolymeric D-glucuronans on partial acid hydrolysis[5]. We report herein the extrinsic Cotton effects exhibited by these polysaccharides, which suggest that they adopt different secondary structures in solution.

Metachromatic binding of Methylene Blue to Polymer I was observed, but the polymer-dye complex did not exhibit a Cotton effect over a wide range of P/D values tested (0.5–10). In contrast, complexes of Polymer II and Methylene Blue showed both metachromatic shifts (Fig. 1, upper panel), and a c.d. doublet with a

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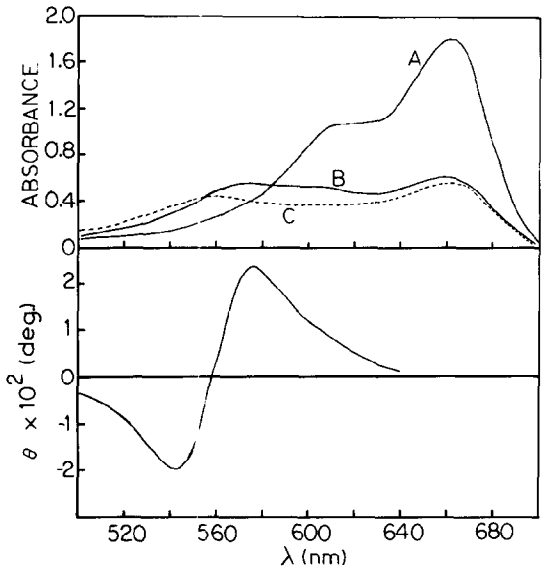

Fig. 1. Absorption spectra (upper panel) and c.d. spectrum (lower panel) of Methylene Blue–polymer complexes. Upper panel: A, Methylene Blue alone; B, Methylene Blue–polymer I complex; and C, Methylene Blue–polymer II complex. P/D ratios were 1.0 for both polymers. Lower panel: c.d. spectrum of Polymer II–Methylene Blue complex at a P/D ratio of 1.0.

TABLE I

CIRCULAR DICHROISM PARAMETERS OF POLYMER II-METHYLENE BLUE COMPLEXES

P/D[a]	*Positive band (1)*		*Cross-over (nm)*	*Negative band (2)*		*Ratio of positive to negative ellipticity (±)*
	Wavelength (nm)	*Molar ellipticity (degrees/dmol of glycuronic acid residues × 10^{-3})*		*Wavelength (nm)*	*Molar ellipticity (degrees/dmol of glycuronic acid residues × 10^{-3})*	
0.7	568	+46.6	548	532	−37.3	1.25
1.0	574	+56.8	557	543	−48.3	1.18
1.3	575	+50.7	558	544	−42.6	1.19
1.7	575	+45.6	558	545	−36.2	1.26
2.2	575	+37.0	558	545	−29.4	1.26
3.3	575	+25.4	558	545	−19.6	1.30
11	575	+4.1	558	545	−3.0	1.36
16	575	+1.2	558	545	−0.8	1.55
22	575	+0.3	558	545		

[a]P/D is the molar ratio of glycosyl residues to Methylene Blue molecules.

positive band centered at 575 nm and a negative band centered at 545 nm (Fig. 1, lower panel). The magnitude of these effects was similar to that of Polymer II from mycelial or yeast walls. The induced c.d. thus allows a ready distinction between D-glucuronans I and II. The absence of a Cotton effect with Polymer I could be due to shorter chain-length of the homopolymeric segments or to differences in the

linkage of the glycosyluronic acid residues as compared with Polymer II. At P/D > 1, the spectrum retained approximately the same shape (Table I), but the effect was diminished, presumably because of the distribution of the dye on excess sites[4]. The shape of the induced c.d. spectrum suggests a left-handed helical array of Methylene Blue molecules in the polymer-dye complex[4]. The retention of a spectrum of essentially the same shape at higher P/D values indicates a uniformity of dye-binding sites along the polysaccharide chain[4]. The significance of the preferred secondary structure of Polymer II in its electrostatic interaction with the positively charged chitin–chitosan complex of the fungal wall remains to be elucidated.

EXPERIMENTAL

Preparation of polysaccharides. — Polysaccharides were extracted from purified cell walls with 6M lithium chloride or M potassium hydroxide, and purified by DEAE-Sephadex chromatography as described elsewhere[5]. Uronic acid content was estimated by the carbazole method of Bitter and Muir[6] with D-glucurono-1,5-lactone as standard.

Circular dichroism spectra. — All spectra were recorded at 25°, with a Cary 60 CD spectropolarimeter having a Cary 6001 CD attachment. For the induced c.d. spectrum, the optical cell had a 1-cm path length and contained 2.7 mL of Methylene Blue (15.7μM) in mM 3-(*N*-morpholino)propanesulfonic acid (MOPS)–potassium hydroxide, pH 7.0. Polysaccharide solutions (1 mg/mL) were added in μL additions with 2 min of stirring. Absorption spectra were recorded with a Beckman Model 24 spectrophotometer.

REFERENCES

1 E. R. BLOUT AND L. STRYER, *Proc. Natl. Acad. Sci. U.S.A.*, 45 (1959) 1591–1593.
2 L. STRYER, Dissertation, Harvard Medical School, Boston, MA, 1961.
3 A. L. STONE, *Biopolymers*, 11 (1972) 2625–2631.
4 A. L. STONE, *Methods Carbohydr. Chem.*, 7 (1976) 120–138.
5 J. M. DOW, D. DARNALL, AND V. D. VILLA, *J. Bacteriol.*, 155 (1983) 1088–1093.
6 T. BITTER AND H. M. MUIR, *Anal. Biochem.*, 4 (1962) 330–334.