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Characteristics and Specificity of the Interaction of a Fluorochrome from Aniline Blue (Sirofluor) with Polysaccharides

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SUMMARY

The effects of polysaccharide structure and environment on the formation of fluorescent complexes between the polysaccharide and a fluorochrome (4,4'-[carbonylbis(benzene-4,1-diyl)bis(imino)]bisbenzenesulfonic acid (Sirofluor) isolated from the triarylmethane dye, aniline blue, have been studied. Amongst the wide range of water-soluble polysaccharides tested, fluorescent complexes are formed only with glucans, the strongest fluorescence being obtained with linear (1→3)-β-D-glucans and with linear (1→3)-β-D-glucans bearing single glucose residues attached at the 6-position. The fluorescence of complexes formed with water-insoluble polysaccharides depends on the ionic environment as well as the polysaccharide structure. (1→3)-β-D-Glucans form strongly fluorescent complexes in the dry state and in the presence of water or phosphate buffer. Various cellulose ((1→4)-β-D-glucan) samples form strongly fluorescent complexes in the dry state and in the presence of phosphate buffer, but are significantly reduced in the presence of water alone.

The fluorescence characteristics reported by Wood & Fulcher (1984) for a range of polysaccharides in alkaline solutions containing aniline blue are in general agreement with the results reported here.

INTRODUCTION

The French botanist Mangin in 1890 discovered that the sulfonated triarylmethane dye, aniline blue (C.I. 42755), was apparently a specific stain for callose in tissues of higher plants and some fungi. Since then this dye has been used extensively in histological studies on the distribution and physiology of callose (Fincher & Stone, 1981). Arens (1949) and later Fidalgo (1954), Currier & Strugger (1956) and Currier (1957) showed that aqueous alkaline aniline blue induced a bright yellow fluorescence with callose when the complex was irradiated with ultraviolet light. The distribution of fluorescence in tissue sections treated with the aniline blue fluorochrome is the same as that observed when aniline blue was used as a dye but the sensitivity of the fluorescence method is greater and there is better contrast between callose deposits and other tissue components (Currier, 1957; Eschrich & Currier, 1964; Smith & McCully, 1978*a*,*b*).

The fluorochrome from commercial aniline blue has been partially purified by chromatography on paper (Arnold, 1956; Kling, 1958) and silica gel (Smith & McCully, 1978*a*). Recently sequential cellulose and silica gel chromatography was used by Evans & Hoyne (1982) to isolate the fluorochrome in an analytically pure form which was characterised by spectroscopy and by synthesis as 4,4′-[carbonylbis-(benzene-4,1-diyl)bis(imino)]bisbenzenesulfonic acid (Sirofluor) (Fig. 1). Two earlier studies have been made on the specificity of complex formation between the aniline blue fluorochrome and polysaccharides, one with an unfractionated fluorochrome mixture (Faulkner *et al.*, 1973) and the other using a partially-purified fluorochrome (Smith & McCully, 1978*a*). In this paper the specificity of complexing of Sirofluor with polysaccharides both in solution at pH 8·5 and in the solid state is described.

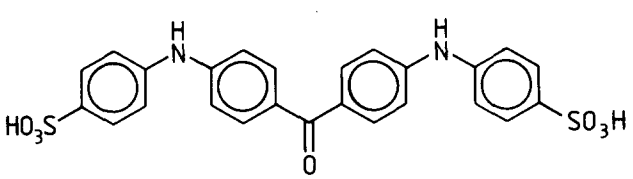

Fig. 1. Structure of the aniline blue fluorochrome.

METHODS

General

Fluorescence excitation and emission spectra were obtained with a Perkin–Elmer MPF-2L fluorescence spectrophotometer fitted with an R-446 photomultiplier. The aniline blue fluorochrome was isolated by the method of Evans & Hoyne (1982). A synthetic sample was obtained by sulfonation of 4,4′-bis(phenylamino)benzophenone (Evans & Hoyne, 1982). The sources and main structural features of the polysaccharides examined for complexing are listed in Table 1.

Fluorescence studies

Fluorescence excitation and emission spectra were measured relative to the fluorescence emission (arbitrary value: 100) at 480 nm of sodium 4-(3′-phenyl-2′-pyrazolin-1′-yl)benzenesulfonate (1 μg/ml) when excited at 380 nm in aqueous solution (Evans *et al.*, 1976), and were not corrected for wavelength dependence of the photomultiplier response.

The following standard conditions for the Sirofluor–polysaccharide systems were selected arbitrarily: Sirofluor concentration $1{\cdot}6 \times 10^{-6}$ M; polysaccharide concentration 2 mg/ml in 0·1 M phosphate buffer at pH 8·5 and 25°C.

The shapes of the fluorescence spectra were similar for all polysaccharides and peak heights gave an approximate measure of relative fluorescence intensities. Fluorescence intensities of solid samples could not be quantitated because of differences in the scatter between samples and because some polysaccharides formed gels in the presence of water. The solid state Sirofluor–polysaccharide complexes were prepared by adding a solution of the Sirofluor ($1{\cdot}6 \times 10^{-6}$ M) in water or phosphate buffer (400 μl) to the polysaccharide (50 mg) followed by careful, thorough mixing. Samples were dried under reduced pressure at 30°C in a rotary evaporator. Both wet and dry samples were exposed to light of wavelength 300–400 nm and the fluorescence emission assessed visually as described in Tables 2 and 3.

TABLE 1
Relative Fluorescence of Sirofluor Complexes with Water-soluble Polysaccharides

Polysaccharide	*Fluorescence*			*Source*	*Supplier*	*References*
	Relative fluorescence	*Maxima* λ_{ex}	λ_{em}			
Alginic acid, Na salt	0			*Macrocystis pyrifera*	Sigma	–
Arabinan	0			–	Koch-Light	–
Arabino-(1→3), (1→6)-β-galactan	0			Larchwood	Sigma	–
Arabino-(1→3), (1→6)-β-galactan-peptide	0			Wheat endosperm	Laboratory prep.	Anderson *et al.* (1977)
Arabino-(1→4)-β-xylan	0			Wheat endosperm	Laboratory prep.	Fincher *et al.* (1974)
Laminaritriose	0			–	Laboratory prep.	–
Cellulose, *O*-hydroxyethyl	0			–	Tokyo Kasei	–
Cellulose, *O*-carboxymethyl	0			–	ICI Ltd	–
Dextrans[a]	0			*Leuconostoc mesenteroides* *Betabacterium vermiforme* *Streptococcus mutans*	Dr G. Walker	–
Gum, acacia	0			*Acacia* sp.	NSW Government Stores	–
Ecklonia-laminarin	0			*Ecklonia radiata*	Dr A. E. Clarke	–
Pectin	0			Citrus fruit	Sigma	–
(1→2)-β-Glucan	0			*Rhizobium meliloti*	Prof. L. P. T. M. Zevenhuisen	Zevenhuisen & Scholten-Koerselman (1979)
Pullulan	0			*Aureobasidium (Pullularia) pullulans*	Sigma	–
Cyclomalto-hexaose	0·04	380	480	–	Sigma	–
Gum, guar	0·1	400	500	*Cyanopsis tetragonolobus*	Sigma	–
Barley β-glucan	0·2	380	480	Barley endosperm	Biocon	Woodward *et al.* (1983)
Gum, locust bean	0·2	395	490	*Ceratonia siliqua*	Sigma	–
Barley β-glucan	0·3	395	490	Barley endosperm	Dr G. B. Fincher	Woodward *et al.* (1983)
Cyclomalto-octaose	0·3	380	480	–	Sigma	–
Ecklonia-glucan	0·4	395	480	*Ecklonia radiata*	Dr P. A. Sullivan	Ram *et al.* (1981)
Isolichenin	0·4	395	470	*Cetraria islandica*	Prof. D. J. Manners	Chanda *et al.* (1957)

Rhodymanan	0·4	395	495	*Palmaria (Rhodymenia) palmata*	Prof. B. Howard	Bjorndal *et al.* (1965)
Yeast mannan	0·5	395	500	Yeast (baker's)	Sigma	–
(1→3)-β-Oligoglucosides, $\overline{DP}$ 8·2	0·6	395	495	Curdlan hydrolysate	Dr K. Ogawa	Ogawa *et al.* (1973)
Cyclomalto-heptaose	0·6	380	480	–	Sigma	–
Amylopectin	1·7	380	480	Potato	Calbiochem	–
Amylose	1·9	380	480	Potato	Calbiochem	–
Amylose	1·9	380	480	Potato, type III	Sigma	–
Eisenia-laminarin	2·0	380	480	*Eisenia arborea*	Tokyo Kasei	Maeda & Nisizawa (1968) Usui *et al.* (1979)
Amylose	2·1	380	480	Potato	BDH	–
Amylopectin, high molecular weight	2·6	380	480	Waxy rice	Mr A. B. Blakeney	–
Glycogen	2·6	380	480	Oyster, type II	Sigma	–
Glycogen	3·0	380	480	Liver	Pfanstiehl	–
Amylopectin, low molecular weight	3·0	380	480	Waxy rice	Mr A. B. Blakeney	–
(1→3)-β-Oligoglucosides, $\overline{DP}$ 11·0	3·4	395	495	Curdlan hydrolysate	Dr K. Ogawa	Ogawa *et al.* (1973)
Pustulan	4·0	395	475	*Pustulata papullosa*	Calbiochem	–
(1→3)-β-Oligoglucosides (unfractionated)	4·6	395	495	Curdlan hydrolysate	Dr K. Ogawa	Ogawa *et al.* (1973)
Pustulan	4·8	395	495	*Lasallia (Umbilicaria) pustulata*	Prof. B. Lindberg	Lindberg & McPherson (1954)
Lichenin	5·7	395	490	*Cetraria islandica*	Prof. D. J. Manners	Fleming & Manners (1966)
Laminaria-laminarin	6·9	395	495	*Laminaria* sp.	Tokyo Kasei	–
Auricularia-glucan	8·3	395	495	*Auricularia auricula-judae*	Prof. A. Misaki	Sone *et al.* (1978)
Claviceps-glucan	8·4	395	490	*Claviceps fusiformis*	Dr K. W. Buck	Buck *et al.* (1968)
(1→3)-β-Oligoglucosides, $\overline{DP}$ 13·9	9·1	395	495	Curdlan hydrolysate	Dr K. Ogawa	Ogawa *et al.* (1973)
Lichenin	10	395	495	*Cetraria islandica*	Laboratory prep.	Clarke & Stone (1965) Moore & Stone (1972)
Scleroglucan	11	395	490	*Sclerotium glucanicum*	Pillsbury Co.	Johnson *et al.* (1963)
Lichenin	12·2			*Cetraria islandica*	Koch-Light	–
Laminaria-laminarin	20	395	495	*Laminaria* sp.	Institute of Seaweed Research	–

TABLE 1 (*Continued*)

Polysaccharide	*Fluorescence*			*Source*	*Supplier*	*References*
	Relative fluorescence	*Maxima*				
		λ_{ex}	λ_{em}			
Laminaria-laminarin	21	395	495	*Laminaria digitata*	US Biochem. Corp.	–
Pachyman, *O*-carboxymethyl, *DS* 0·3	25	395	495	–	Laboratory prep.	Stone (1972)
Yeast glucan	26	395	495	Oriental baker's yeast, Fr S3P	Prof. A. Misaki	Misaki *et al.* (1968)
Sclerotan	26	395	495	*Sclerotinia libertiana*	Dr S. Oi	–
Iso-sclerotan	29	395	495	*Sclerotinia libertiana*	Dr S. Oi	Oi *et al.* (1966)
Pestalotia-glucan	31	395	490	*Pestalotia* sp.	Prof. A. Misaki	–
Lentinan	31	395	495	*Lentinus edodes*	Ajinomoto Co.	Hamuro *et al.* (1971) Saitô *et al.* (1977*b*)
Schizophyllan	33	395	495	*Schizophyllum commune*	Prof. A. Misaki	Kikumoto *et al.* (1970)
Auricularia glucan II, polyalcohol[b]	40	395	490	*Auricularia auricula-judae*	Prof. A. Misaki	Sone *et al.* (1978)

[a] The (1→6)-linkage content of the dextran samples was between 66 and 95%.
[b] Complex fluorescence is reduced by light.
Suppliers addresses: ICI Ltd, Stevenston, Ayrshire, Scotland; US Biochem. Corp., PO Box 22400, Cleveland, Ohio, USA; Sigma Chemical Co., St Louis, Mo, USA; Koch Light Laboratories Ltd, Colnbrook, Buckinghamshire, England; Tokyo Kasei Ltd, 3-4-9 Ninonkashi-Honcho, Chuo-ku, Tokyo, Japan; Biocon (Aust.) Pty Ltd, 31 Wadhurst Drive, Knoxfield, Victoria, Aust.; Ajinomoto Co., 214 Maeda-cho, Totsuka-ku, Yokohama, Japan; Calbiochem-Behring Australia Pty Ltd, PO Box 37, Carlingford, NSW, Aust.; BDH Ltd, Poole, Dorset, UK; Pfanstiehl Laboratories Inc., 1219 Glen Rock Avenue, Waukegan, Ill., USA; Pillsbury Co., Minneapolis, Minnesota, USA; Institute of Seaweed Research, Inveresk, Midlothian, Scotland.

TABLE 2
Fluorescence of Sirofluor-Polysaccharide Complexes in the Solid State

Polysaccharide	*Fluorescence emission*[a]		
	Dry	*Wet*	
		Water	*Buffer*[b]
Cellulose CF-11 (Whatman)	3	1	3
Amylopectin	3	1	3
Paramylon	3	3	3
Curdlan	3	3	3
Pachyman	3	3	3
Avicel (microcrystalline cellulose)	3	2	3

[a] 3, Strong fluorescence; 2, moderate fluorescence; 1, weak fluorescence; 0, negligible fluorescence. (When the sample is exposed to radiation of wavelength 300–400 nm.)
[b] 0·1 M phosphate buffer at pH 8·5.

TABLE 3
Effect of Phosphate Buffer Concentration on the Fluorescence of Sirofluor-Cellulose CF-11 Mixtures

Buffer concentration (M)	*Fluorescence emission*[a]
1·0	3
0·1	3
0·01	2
0·001	1
0·0001	0

[a] See Table 2 for irradiation conditions and an explanation of the fluorescence emission intensities.

RESULTS

Characteristics of Sirofluor-polysaccharide complexes

The excitation and emission spectra of the fluorescence induced by complexing the purified Sirofluor with amylopectin (Fig. 2) and other polysaccharides (Table 1) showed maxima in the range 380–395 nm and 470–500 nm respectively. The synthetic Sirofluor showed the same fluorescence excitation and emission maxima and similar relative fluorescence intensities. These characteristics correspond with those reported by Smith & McCully (1978*a*) and Wood & Fulcher (1983) for the fluorescence of complexes of partially purified and unpurified aniline blue with various β-glucans.

The complex between Sirofluor and amylopectin had moderately intense fluorescence and although the intensity is not as high as with other soluble polysaccharides, such as *Laminaria*-laminarin, the ready availability of amylopectin makes it a useful model.

Complexing with amylopectin is independent of pH in the range 3–10 but between pH 10 and 13 there is a marked decrease in intensity

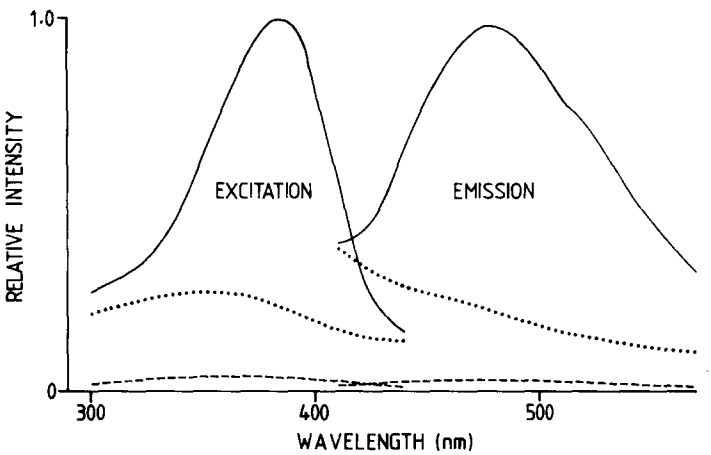

Fig. 2. Fluorescence excitation and emission spectra of the Sirofluor-amylopectin and a mixture of these two substances at pH 8 in 0·007 M phosphate buffer. Sirofluor concentration $1{\cdot}6 \times 10^{-6}$ M; amylopectin concentration 4·7 mg/ml; (——) mixture; (– – – –) Sirofluor; (·····) amylopectin.

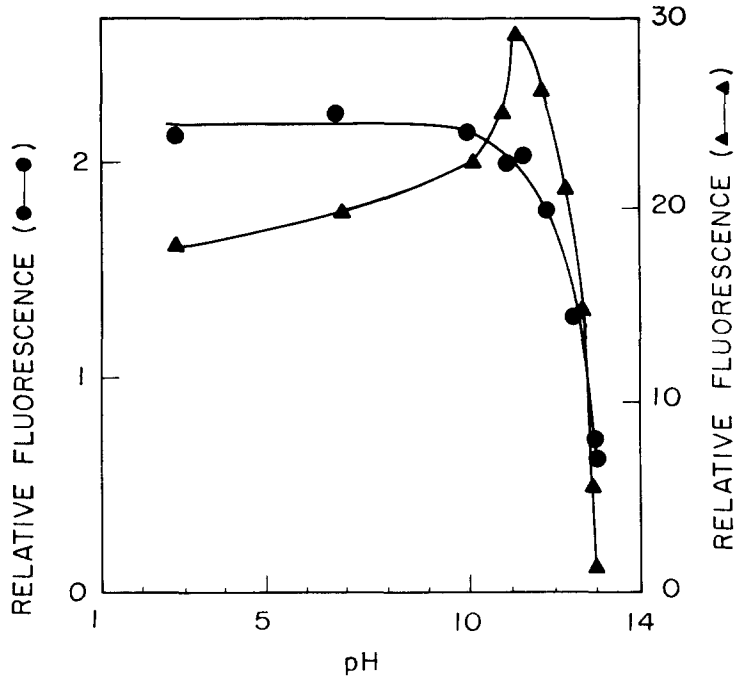

Fig. 3. Dependence of the relative fluorescence of the Sirofluor-amylopectin (●——●) and Sirofluor-*Laminaria*-laminarin (▲——▲) complexes on pH. The following buffers were used: pH 3, 0·1 M glycine-HCl; pH 7, 0·1 M phosphate; pH 10·2-13·0, 0·1 M glycine-NaOH.

(Fig. 3). *Laminaria*-laminarin shows somewhat different behaviour (Fig. 3). There is a small increase in fluorescence intensity as the pH is raised from 3 to 10, then a sharp increase to a maximum at pH 11·5; thereafter, as for amylopectin, the intensity decreases to a very low value at pH 13.

Specificity of complexing with water-soluble polysaccharides

The relative fluorescence and wavelength maxima of excitation and emission for a number of soluble polysaccharides are recorded in Table 1. These data enable some generalisations to be made concerning

the specificity of the complexing interactions:

(i) Induction of fluorescence is shown only by α- or β-glucans, other homo- or hetero-glycans, e.g. (1→3),(1→4)-β-xylan, arabino-(1→4)-β-xylan, arabino-(1→3),(1→6)-β-galactan, pectin were ineffective.

(ii) Not all α-D-glucans induce fluorescence. The (1→4)-α-D-glucan (amylose), the (1→4),(1→6)-α-D-glucans (amylopectin and glycogen) and the cyclic (1→4)-α-D-oligoglucosides induce a weak fluorescence but a group of (1→6)-α-D-glucans (dextrans), the (1→4),(1→6)-α-D-glucan (pullulan) and the (1→3),(1→4)-α-D-glucan (isolichenin) give no fluorescence. Faulkner *et al.* (1973) also found that amylose and amylopectin induced moderate fluorescence with the unpurified aniline blue fluorochrome but that, in addition some dextrans and pullulan were also moderately active.

(iii) The linear (1→3)-β-D-glucans, *Laminaria*-laminarin and the water-soluble (1→3)-β-D-glucan derivative *O*-carboxymethylpachyman induce intense fluorescence.

(iv) The induction of fluorescence with (1→3)-β-oligoglucosides depends on the degree of polymerization ($\overline{DP}$). Thus laminaritriose is inactive, but very weak fluorescence is observed with a $\overline{DP}$ 8·2 oligoglucoside.

The $\overline{DP}$ 13·9 oligoglucoside gives a moderate fluorescence whereas *Laminaria*-laminarin ($\overline{DP} \simeq 20$) gives an intense fluorescence.

(v) A family of β-D-glucans with linear (1→3)-β-D-glucan main chains, bearing single (1→6)-linked β-D-glucosyl substituents at intervals, induced intense fluorescence although to different degrees. Those tested were the β-glucans from *Lentinus edodes*, *Claviceps* sp., *Auricularia auricularia-judae*, *Sclerotinia libertiana*, *Sclerotinia* sp., *Schizophyllum commune* and *Pestalotzia* sp. When the *Auricularia*-glucan is oxidized with periodate and reduced, the molecule is converted to a (1→3)-β-D-glucan bearing acetal-linked glycerol and glycolaldehyde substituents. This derivative showed a greater ability to enhance fluorescence than the parent glucan. Branched glucans in which the main chain contained (1→6)-linked as well as (1→3)-linked glucosyl residues, e.g. the *Eisenia*- and *Ecklonia*-laminarins induce very weak or no fluorescence.

(vi) The (1→6)-β-D-glucan, pustulan, induces a fluorescence with an intensity comparable with that of amylopectin.

(vii) The soluble (1→4)-β-D-glucan derivatives, *O*-carboxymethyl-cellulose and *O*-hydroxyethyl-cellulose, and a (1→2)-β-D-glucan do not induce fluorescence.

(viii) The (1→3),(1→4)-β-D-glucans, lichenin and barley β-glucan, show apparently contradictory behaviour. Barley glucan induces almost no fluorescence whereas the three lichenin samples tested induce a moderately strong fluorescence.

Thus three broad groups of soluble glucans can induce fluorescence by complexing with Sirofluor: the (1→4)-α-D-glucans, some (1→3),-(1→4)-β-D-glucans and the unsubstituted or substituted (1→3)-β-glucans. Of these the (1→3)-β-D-glucans and their derivatives induce the most intense fluorescence but there is a five-fold difference in relative intensity among the samples tested from this group. The differences presumably relate to such factors as degree of polymerization, and degree and nature of substitution of the (1→3)-β-D-glucan chain.

Complexing with insoluble polysaccharides

The relative fluorescence of the complex with some insoluble polysaccharides in the dry state is compared in Table 2 with their corresponding fluorescence when wet. Surprisingly, in the dry state, not only did the (1→3)-β-glucans, paramylon, pachyman and curdlan, induce fluorescence but so also did several cellulose ((1→4)-β-D-glucan) samples. When the dry complexes are wetted with deionized water (pH ~ 5) the fluorescence of the Sirofluor–cellulose mixtures is greatly reduced. However, if phosphate buffer (pH 8·5) is used to wet the Sirofluor–cellulose mixture fluorescence is still observed. This effect could not be reproduced using water adjusted to pH 8·5 and is dependent on the concentration of phosphate (Table 3).

DISCUSSION

The aniline blue fluorochrome, Sirofluor, is one of a number of compounds such as Calcofluor white M2R New (Wood, 1980), 2-*p*-toluidinyl-naphthalene-6-sulfonic acid (TNS) (Kondo *et al.*, 1976) and

Congo Red (Wood & Fulcher, 1978) which complex with polysaccharides to induce intense fluorescence. These compounds show varying degrees of specificity. Calcofluor complexes with (1→4)-β-D-glucans and (1→3)-β-D-glucans (Maeda & Ishida, 1967; Wood & Fulcher, 1978; Wood *et al.*, 1983), TNS with amylose (Nakatani *et al.*, 1977) and the aniline flue fluorochrome gives intense fluorescence with (1→3)-β-D-glucans although other glucans also form weaker complexes.

In no case are the precise details of complex formulation understood so that the specificities cannot at present be explained in molecular terms. Presumably specific sites are present on the polysaccharide chains which immobilise the fluorochrome so that energy received on irradiation is dissipated as a fluorescence emission rather than as enhanced molecular vibration.

The specificity of Sirofluor complexing allows some deductions regarding the structural requirement for complexing. (1→3)-β-D-Glucans exist in both solution (Casu *et al.*, 1966; Ogawa *et al.*, 1972) and the condensed state (Marchessault *et al.*, 1977; Takeda *et al.*, 1978; Marchessault & Deslandes, 1979; Deslandes *et al.*, 1980; Fulton & Atkins, 1980) in an ordered, open helical conformation which may be essential for complexing with Sirofluor. It is known that this conformation is also assumed by substituted (1→3)-β-glucans in solution (Bluhm & Sarko, 1977; Norisuye *et al.*, 1980; Yanaki & Norisuye, 1983) and these polymers also give good complexing. The occurrence of more flexible (1→6)-β-glucosidic linkages in a (1→3)-β-glucan chain, as in the *Eisenia*- and *Ecklonia*-laminarins, would disrupt the ordered conformation and reduce the number of binding sites. These polymers show poor complexing. Model building studies show that Sirofluor, which is a flexible molecule with a length equivalent to a glucosyl tetrasaccharide, could interact specifically with the surface of a (1→3)-β-D-glucan triple helix possibly through hydrophobic regions. This is also suggested by the observation of Evans & Hoyne (1982) that Sirofluor is induced to fluoresce in the presence of organic solvents and the cationic detergent hexadecyltrimethylammonium bromide. The need for a regular conformation for fluorochrome complexing is supported by the observation that fluorescence is lost at pH values greater than 12, conditions under which (1→4)-α-glucans (Erlander *et al.*, 1968) and (1→3)-β-glucans (Ogawa *et al.*, 1973) adopt a random coil conformation. Nakanishi *et al.* (1974) made similar observations

for the aniline blue dye and Ogawa *et al.* (1972, 1973) showed that Congo Red binding to curdlan was also abolished as the pH was raised above 12. The increased intensity of Sirofluor–*Laminaria*-laminarin complexing in the pH 10–11·5 region must relate to the generation of new chain conformations capable of stronger interactions with Sirofluor. The nature of these conformations remains to be determined.

(1→3)-β-Glucans of $DP < 20$, do not adopt helical conformations in dilute alkaline solutions (Saitô *et al.*, 1977*a*) although the disordered (random coil) forms may take up an ordered structure in the presence of Congo Red (Ogawa & Hatano, 1978). Thus the poor complexing of (1→3)-β-oligoglucosides is also consistent with the presumed requirement for an ordered conformation of the polymer. Although the (1→3),(1→4)-β-glucans, lichenin and barley glucan, are both linear molecules with ~30% (1→3) and ~70% (1→4) linkages, data derived from chemical and enzymic degradation suggests that differences in the proportion of **-G4G4G3-** and **-G4G4G4G3-** sequences could be responsible for the differences in fluorescence characteristics. Lichenin has a higher proportion of shorter sequences so that regular fluorochrome binding conformations may exist along segments of the lichenin chain. Appropriate conformations apparently do not occur in barley (1→3),(1→4)-β-glucan where the randomly arranged **-G4G4G3-** and **-G4G4G4G3-** sequences (Staudte *et al.*, 1983) are interspersed with occasional sequences containing up to 12 (1→4)-linked residues (Woodward *et al.*, 1983).

The induction of moderate fluorescence by amylose and amylopectin, previously noted by Faulkner *et al.* (1973), and a very weak fluorescence by cyclic (1→4)-α-D-oligoglucosides, may relate to regular helical conformations adopted by the (1→4)-α-glucan parts of the polymers and to the ability of the cyclic oligoglucosides to form stable inclusion complexes with ligands of appropriate dimensions (Kondo *et al.*, 1976; Rohrbach & Wojcik, 1981).

Sirofluor complexes well with (1→3)-β-glucans such as paramylon, pachyman and curdlan in the condensed state, but as noted by Faulkner *et al.* (1973) and Smith and McCully (1978*a*), cellulose in 0·067 M phosphate buffer (pH 8·5), also induces fluorescence when used as recommended by Eschrich & Currier (1964). Our results also show that Sirofluor complexes with both types of β-glucan and elicits fluorescence in the dry state.

An important observation was that in the wet state the fluorescence of cellulose is progressively diminished to very low levels as the ionic strength of the wetting medium is decreased. At present it is not possible to account for these observations in terms of differences in the mechanism and stereochemistry of binding of the aniline blue fluorochrome to (1→3)-β-glucans and cellulose. However, they have a practical application in choosing conditions to increase the selectivity and contrast of Sirofluor fluorescence in botanical sections containing both cellulosic and (1→3)-β-glucan regions (Stone *et al.*, 1983).

In summary, it is clear that Sirofluor has a high specificity for binding to (1→3)-β-glucans in solution and this ability may be exploited in a number of ways comparable to the use of the iodine–starch complex, for example, in quantitation of (1→3)-β-glucans in solution and in studies on the mechanism of their depolymerization by enzymes. Further studies on the Sirofluor–polysaccharide complex both in solution and the condensed state are in progress to define the nature of the interaction.

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