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Complex Formation of (1→3)- β -D-Glucan with Some Azo Dyes

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Abstract

The complex formations of a (1→3)- β -D-glucan with seven direct azo dyes having the same backbone bisazo structure but different side groups were studied by means of visible spectroscopy. All the dyes showed red shifts (color change) by the addition of the glucan in an alkaline solution where the glucan takes an ordered conformation, indicating complex formations. The degree of the red shifts depended on the hydrophobicities of the side group at the phenyl groups of the dye backbone structure: methyl > hydrogen > methoxy. Amino groups attached to the backbone naphthalene groups contributed to the red shifts more than hydroxyl groups did. Whereas, a sodium sulfonate group was considered to disturb the complex formation.

Introduction

It has been well known that many benzidine direct azo dyes, typified by congo red (Color Index Direct Red 28) whose chemical structure is shown in Table 1, form complexes with cellulose, (1→4)- β -D-glucan. In the backbone structure of these bisazo dyes all the rings of naphthalene and phenyl, and azo groups are arranged in a flat plane over which π electrons are delocalized¹⁾. The π electrons and substituted amino (or hydroxyl) groups make hydrogen bonds with the hydroxyl groups of cellulose resulting in the complex formations.

We have reported that curdlan, a linear (1→3)-linked β -D-glucose polymer, made a complex with congo red in a dilute alkaline solution^{2,3)} where the polysaccharide took an ordered conformation⁴⁾, and that the driving force for the complex formation is entropic in nature which may occur the decrease of "iceberg formation" surrounding the glucan chain⁵⁾. Our finding of the complex formation²⁾ has actually been utilized as a means of examining the antitumor activities not only of curdlan but also of other (1→3)- β -D-glucans. Our interests are to find suitable dyes other than congo red for the examination the glucan's activities and to elucidate how the complex is formed. In this paper we report preliminary results obtained with the complex formations of curdlan with seven bisazo dyes of the same backbone chemical structure but different side groups in dilute alkaline solutions.

Materials and Methods

Curdlan-type polysaccharide 13140, a linear (1→3)- β -D-glucan, was supplied by Takeda Chemical Industries Ltd. The number average degree of polymerization of the glucan was determined to be 462 by Nakanishi *et al.*⁶⁾ Seven direct azo dyes having the same backbone chemical structure were used. As reported previously⁶⁾ the purity of congo red (Color Index Direct Red 28, mol. wt. 697), purchased from Wako Chemical Co. Ltd., was 82%. Azo Blue (C.I. Direct Violet 39, mol. wt. 727), the purity of which was 44%, was purchased from Tokyo Kasei Co. Ltd. Brilliant Blue (C.I. Direct Blue 15, mol. wt. 993) having 40% purity was purchased from Sigma Co. Ltd. Whereas, purities of Benzopurpurine 4B (C.I. Direct Red 2, mol. wt. 725) purchased from Wako Chemical Co. Ltd., Benzopurpurine 10B (C.I. Direct Red 7, mol. wt. 757) supplied by Bayer Japan Ltd., Direct Blue 2B (C.I. Direct Blue 6, mol. wt. 933) supplied by Nippon Kayaku Co., and Trypan Blue (C.I. Direct Blue 14, mol. wt. 961) purchased from Wako Chemical Co. Ltd. were not known. Chemical structures of all the dyes are shown in Table 1. All the dyes can be classified into three groups according to the side groups attached to the naphthalene groups of the backbone bisazo structure: on the half structure basis, group A has a sodium sulfonate and an amino group; B, a sodium sulfonate and hydroxyl group; and C, two sodium sulfonates, an amino group and a hydroxyl group. Each group is further classified according to the side groups (hydrogen, methyl, or methoxy groups) attached to the phenyl groups of each backbone. In the present study we disregarded the influence of the impurities in the dyes on the formation of the complexes, since major components were sodium chloride and sodium sulfate which do not affect the rates of complex formations, in particular, at such low concentrations as $<4 \times 10^{-4}$ M for sodium chloride and $<2 \times 10^{-4}$ M for sodium sulfate estimated in this experiment. Visible absorption spectra were measured with a Hitachi U-3210 Spectrophotometer at 25°C.

Results and Discussion

As reported in the previous study on the glucan-congo red complex formation²⁾, when the glucan concentration increased the absorption maximum of the dye shifted to longer wavelength until reaching the longest value which was regarded as the absorption maximum of the complex, $\lambda_{\max}^{\text{comp}}$. Figure 1 shows the longest red shifts of seven azo dyes caused by the addition of the (1→3)- β -D-glucan in a 0.1M NaOH solution at 25°C where the glucan takes an ordered conformation⁴⁾. These facts suggest that all the dyes studied form complexes with the glucan. All the spectra recorded with solid lines in Fig. 1 are of the respective complexes, and the absorption maximum of each complex is shown in Table 1 together with that of the corresponding free dye, $\lambda_{\max}^{\text{dye}}$. The absorption maxima of the dyes of groups A and B showed distinguishable red shifts, whereas, each dye of group C showed a very small red shift.

From Fig. 1 and Table 1, effect of side group on the complex formation can be discussed. In the dyes of group A the degree of red shift ($\Delta\lambda$) is $\text{CH}_3 > \text{H} > \text{OCH}_3$. Since this tendency agrees with the hydrophobicity of the side groups, the hydrophobicity contributes to the red shifts occurring by the complex formation. Based on the circular dichroism study of the glucan-congo red complex we have reported that the complex is stabilized by strong hydrogen bonds and hy-

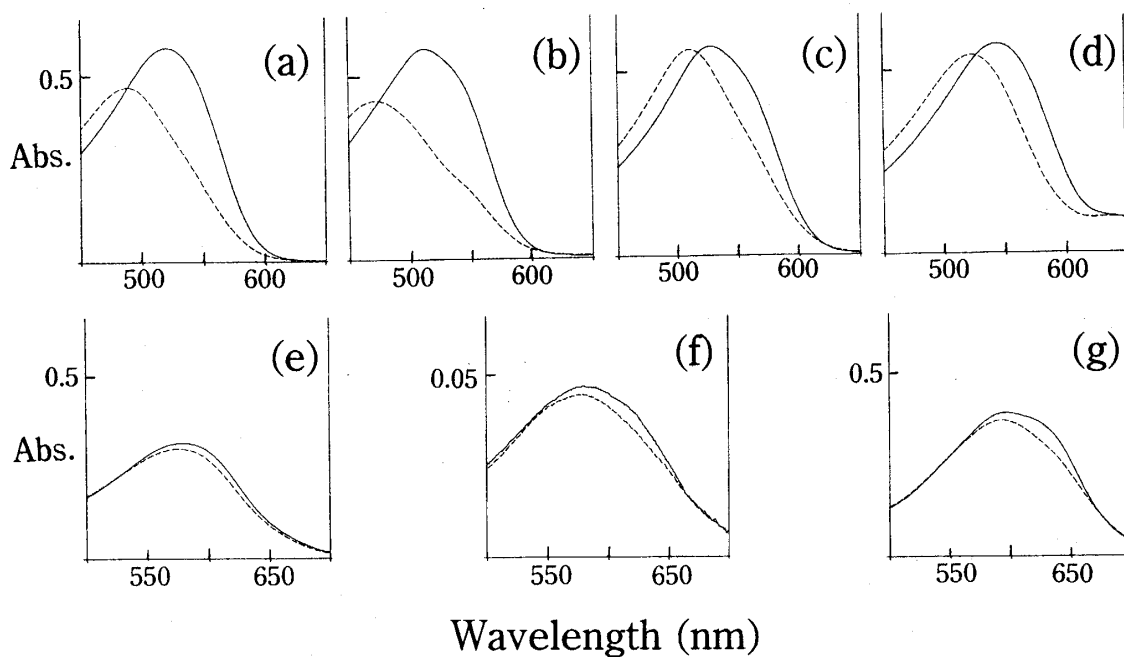
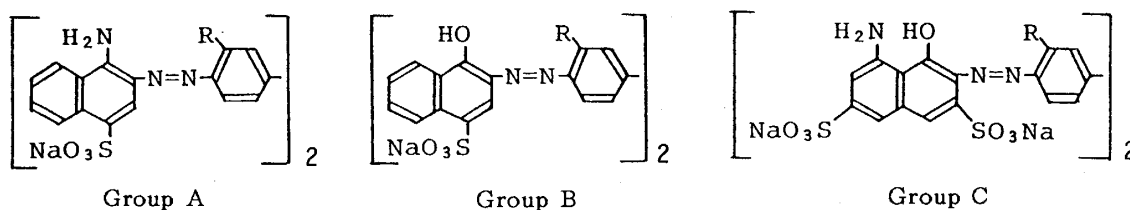


Fig 1. Visible absorption spectra of complexes of (1→3)- β -D-glucan with seven azo dyes (solid lines) and the dyes (dashed lines) in 0.1M aqueous NaOH solutions at 25°C. (a) C.I. Direct Red 28 (congo red); (b) Red 2; (c) Red 7; (d) Violet 39; (e) Blue 6; (f) Blue 14; (g) Blue 15. Total glucan concentration denoted by glucose residue concentration, and those of dyes, are shown in Table 1. Light path is 10mm.

Table 1 Absorption maxima of free dyes and the complexes



Group	R	C.I. Direct	Dye Conc ⁿ .	$\lambda_{\max}^{\text{dye}}$ (nm)	$\lambda_{\max}^{\text{comp}}$ (nm)	$\Delta \lambda$ (nm) ^b
A	H	Red 28	$1.18 \times 10^{-5} \text{M}$	489	520	31
	CH ₃	Red 2	15mg/l	472	513	41
	OCH ₃	Red 7	13mg/l	510	528	18
B	CH ₃	Violet 39	$0.56 \times 10^{-5} \text{M}$	522	544	22
C	H	Blue 6	24mg/l	576	579	3
	CH ₃	Blue 14	4mg/l	579	582	3
	OCH ₃	Blue 15	$0.44 \times 10^{-5} \text{M}$	593	599	6

ⁿThe dyes whose concentrations are denoted by the unit mg/l are of unknown purity. The glucan concentration denoted by glucose residue concentration, R_0 , = $5.00 \times 10^{-2} \text{M}$

^bThe difference of wavelength of absorption maximum between dye and complex

dophobic interactions³⁾. The hydrophobic bonds may present between the glucan and the side groups at the phenyl groups of the backbone structure of each dye. When comparing the red shift of C.I. Direct Red 2 with that of Violet 39, the former ($\Delta\lambda=41\text{nm}$) showed longer red shift than the latter (22nm). The structural difference between the two are in the side group attached to the backbone phenyl groups, amino groups in the former and hydroxyl groups in the latter. This fact suggests that a hydrogen bond is formed between these side groups and the glucan molecule to make the complex and that the contribution of the amino groups to the color change is larger than that of the hydroxyl groups. A sodium sulfonate group may disturb the complex formation since the group C dye having two sulfonates attached to the naphthalene groups showed only a small red shift.

For the examination of the antitumore activities of (1 \rightarrow 3)- β -D-glucans, Direct Red 2, Red 7, or Violet 39 may also be available besides congo red since the red shift (color change) of these dyes that occurred by the addition of the glucan were comparable with that of congo red (Table 1).

Acknowledgments

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