CHEMISTRY LETTERS, PP. 689-692, 1972. Published by the Chemical Society of Japan

COMPLEX OF GEL-FORMING β-1,3-D-GLUCAN WITH CONGORED IN ALKALINE SOLUTION

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Complex formation of gel-forming β-1,3-D-glucan ($\overline{DP}n$ = 500) with congored in alkaline solution was studied by measurements of visible absorption spectra. Molecular absorption coefficient of the complex at 520 nm was determined to be 2.98×10^4 per congored and formation constant of the complex, 6.4×10^5 M^{-1}, in 0.1 N sodium hydroxide solution at 25°C.

It has been shown[1)2)] that the bacterial β-1,3-D-glucan, isolated by Harada et al.[3)-5)] is soluble in aqueous alkaline but not in neutral or acid solution, and that it forms a gel when its aqueous suspension (at pH<12) is heated above 54°C. In our previous paper[6)], measurements of optical rotatory dispersion, viscosity, and flow birefringence indicated that, in low alkaline solutions, gel-forming β-1,3-D-glucan took an ordered structure which was probably a helical conformation, and in high alkaline solutions, a random coil. Conformational transition of the glucan occurred in the range of sodium hydroxide concentration from 0.19 to 0.24 N[6)].

Congored has been known to form complexes with several α-1,4-D-glucans. Sensse and Cramer[7)] studied complexes of this dye with amylose and cyclodextrins by measuring visible absorption spectra and the optical rotatory dispersion, and Takeo and Kuge[8)] by circular dichroism measurements. In the present paper, we wish to report a complex of β-1,3-D-glucan with congored in alkaline solution[9)].

As β-1,3-D-glucan, 13140 polysaccharide, one of curdlan type polysaccharides[10)], supplied by Takeda Chemical Industries Ltd. was used. This polysaccharide was reported to be a linear polymer of glucose linked by β-1,3-glucosidic linkage[10)]. Number average degree of polymerization of the glucan was determined to be 500 by the modified Somogyi-Nelson method[11)] using laminaribiose as a standard. Congored was a product of Chroma, Stuttgart. Visible absorption spectra were measured by a Hitachi Model 323 Recording Spectrophotometer at 25°C.

Fig. 1 shows visible absorption spectra of congored in 0.10 and 0.30 N sodium hydroxide solutions in the presence and absence of the gel-forming β-1,3-D-glucans. At low alkaline concentration (0.10 N), absorption maximum (λ_{max}) of congored (489 nm) is largely shifted to longer wavelength (520 nm) in the presence of the glucan, while, at high alkaline concentration (0.30 N), the λ_{max} is little shifted (from 486 to 489 nm). Relationship between the λ_{max} and sodium hydroxide concentration is shown in Fig. 2. The sharp change of λ_{max} of the glucan-congored solution is

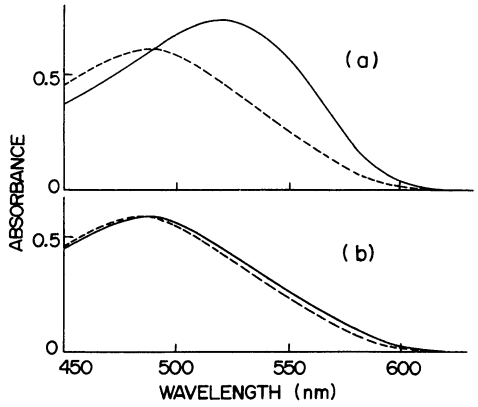

Fig. 1. Visible absorption spectra of congored in alkaline solution in the presence (solid lines) and in the absence (dotted lines) of the glucan at 25°C; (a) 0.10 and (b) 0.30 N sodium hydroxide solutions. Total concentrations of the glucan denoted in residue concentration, $R_0=3.1\times10^{-2}$M, and of congored, $D_0=2.3\times10^{-5}$M.

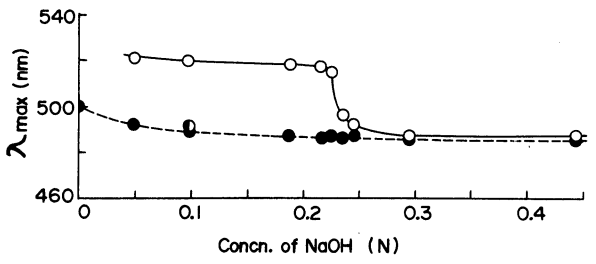

Fig. 2. Dependence of the absorption maximum (λ_{max}) of congored in the presence and absence of the glucans on alkaline concentration at 25°C; $R_0=3.1\times10^{-2}$M, and $D_0=2.3\times10^{-5}$M. ○:the gel-forming glucan($\overline{DP}n=500$) -congored, ◐:the water-soluble glucan($\overline{DP}n=17.1$)-congored, and ●:congored only.

observed in a range of sodium hydroxide concentrations from 0.22 to 0.25 N. This range corresponds to the alkaline concentration range where the conformational transition of the glucan occurs as stated previously[6]. When low molecular weight (water soluble) β-1,3-D-glucan[12] is used, red shift of λ_{max} of congored is small as shown in Fig. 2. These results clearly indicate that the gel-forming β-1,3-D -glucan forms complex with congored in low alkaline media where the glucan takes an ordered conformation.

Fig. 3 shows absorption spectra of the glucan-congored systems in 0.10 N sodium hydroxide solution at various concentrations of glucan (R_0: in terms of mole of glucose residues per liter). Absorption maximum was shifted to longer wavelength with increasing R_0, the isosbestic point being at 485 nm. The presence of an isosbestic point indicates that the absorption spectrum of a given solution is the additive form of two individual absorption spectra of two species, i.e. the free dye and the complex, in equilibrium. Dependence of λ_{max} on the concentration ratios of glucan (R_0) to congored (D_0) was shown in Fig. 4. Most experiments were carried out at a constant R_0 of 1×10^{-3}M. However, in order to see effect of glucan concentrations, the other three concentrations of glucan were also adopted. As evidenced in Fig. 4, with increasing R_0/D_0, λ_{max} tends to be shifted to longer wavelength, approaching to a limiting value of 520 nm. Throughout these experiments absorbances of all the glucan-congored solutions having λ_{max} at 520 nm were proportional to the congored concentrations in them, suggesting that congored was completely complexed with the glucan. Thus molecular absorption coefficient of the complex at 520 nm (ε_{520}^{comp}) was estimated to be 2.98×10^4 per mole congored in 0.1 N sodium hydroxide solution at 25°C. That of free congored at 520 nm (ε_{520}^{dye}) was

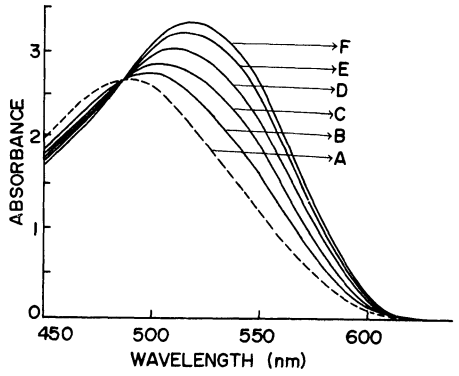

Fig. 3. Effect of increasing concentrations of the glucan on the absorption spectrum of congo-red (D_0; 1.08×10^{-4}M) in 0.10 N sodium hydroxide at 25°C. Spectra are shown at the following concentrations of the glucan (R_0): curve A, zero; B, 0.32×10^{-2}; C, 0.63×10^{-2}; D, 0.95×10^{-2}; E, 1.59×10^{-2}; F, 3.09×10^{-2}M.

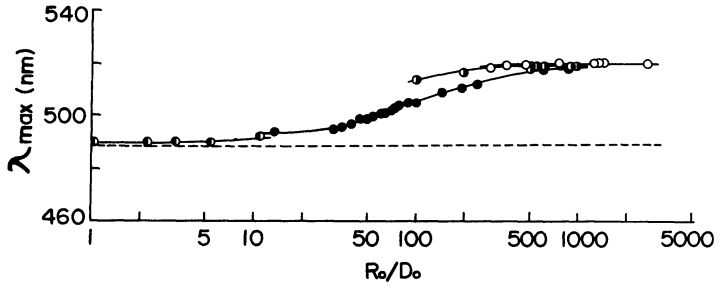

Fig. 4. Change of λ_{max} of congo-red in the presence of the glucan in 0.10 N sodium hydroxide with the concentration ratio, R_0/D_0 at 25°C; values of R_0 are ○ :3.1×10^{-2}, ◑ :1×10^{-2}, ● :1×10^{-3}, and ◐ :3.1×10^{-4}M. Dotted line shows λ_{max} of free congored (489 nm).

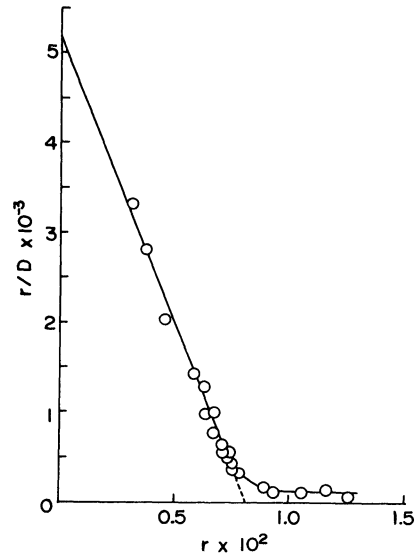

Fig. 5. The relationship between r/D and r at 25°C. Measurements were made in 0.10 N sodium hydroxide solutions, and at a constant R_0 of 1.0×10^{-3}M.

determined to be 1.99×10^4 under the same condition. Assuming that a mole of the dye combines with a glucan segment containing n glucose residues in the complex, the following equilibrium will hold in solution.

Segment + Dye = Complex .....(1)

The formation constant of the complex (K) is

$$K = \frac{C}{SD} = \frac{C}{(S_0 - C)D} \quad \text{.....(2)}$$

where S, D, and C are concentrations of free segment, free dye, and complex, respectively. S_0 is the total concentration of the segment and equals to R_0/n (R_0:

total concentration of glucan expressed in terms of glucose residue). Equation (2) can be written as follows,

$$\frac{r}{D} = \frac{K}{n} - Kr \qquad \text{.....(3)}$$

where r represents the concentration ratio of the complex to total glucan, C/R_0. Fig. 5 shows the relationship between r/D and r: the concentration of the complex (C) was determined from absorbance of the glucan-congored solution at 520 nm by using values of ε_{520}^{comp} and ε_{520}^{dye}. The straight line through the points at low value of r gave $K = 6.4\times10^{5}M^{-1}$, and n = 123. Although no pertinent interpretation of the break near $r = 0.8\times10^{-2}$ is available at present, it may concern with the further binding of the glucan with congored. A similar finding was reported in the complex formation of transfer RNA with ethidium bromide[14].

FOOTNOTES AND REFERENCES

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9) I. Nakanishi, private communication; complex formation of this polysaccharide with several dyes including congored were independently found by Nakanishi, a member of Takeda Chemical Industries Ltd. Details will be published elsewhere.

10) T. Harada, presented at the Fourth International Fermentation Symposium, Kyoto, March, 1972.

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12) This sample was prepared by fractional precipitation of partially degraded glucan which was obtained from formic acid treatment of 13140 polysaccharide[13]. Number average degree of polymerization of this glucan determined to be 17.1 by the modified Somogyi-Nelson method. It was confirmed with measurements of optical rotatory dispersion that this low molecular weight glucan takes a disordered conformation in neutral and alkaline solutions[13].

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(Received June 6, 1972)