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Interaction between Copper Chlorophyllin and Poly(vinyl alcohol)

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Synopsis. The difference spectrum between copper chlorophyllin (Cu-chln) plus poly(vinyl alcohol) (PVA) and Cu-chln alone exhibits a maximum at 652 nm in water. The Benesi-Hildebrand plots give equilibrium constants of 833 $\text{mol}^{-1}\,\text{dm}^3$ for UMR-20H (degree of saponification: 81.7%) and 1820 $\text{mol}^{-1}\,\text{dm}^3$ for UMR-20M (degree of saponification: 63.8%). The Scatchard plot of these data shows the existence of a cooperative binding.

In a biological system, chlorophylls are bound to proteins to form several molecular species called "chlorophyll forms".[1] Complexes between chlorophyll and polymers have been studied as models for the chlorophyll forms.[2] We studied the interaction between copper chlorophyllin (Cu-chln), a model for chlorophyll, and such polymers as poly (riboadenylic acid),[3] ribonucleic acid,[4] poly(*N*-vinylpyrrolidone).[5,6] The results showed that the coordination between the Cu(II) ion of Cu-chln and the oxygen or nitrogen atom of a polymer causes inter- or intramolecular bridging of polymers, whereby the solution viscosity is increased and the rotational correlation time is lengthened.

In this work the author investigated the interaction of Cu-chln with poly(vinyl alcohol) (PVA) having hydroxyl groups as side chains. A relationship is noted between the degree of saponification of PVA and the strength of the interactions.

Experimental

Partially saponified PVA (Unitika Chemical Co, UMR-20L$^{\text{R}}$, degree of saponificaion: 35.0%, UMR-20M®, degree of saponification: 63.8%, UMR-20H®, degree of saponification: 81.7%) were used. Poly(vinyl acetate) was purchased from Wako Pure Chem. Co. Cu-chln was purchased from Nakarai Tesque Co., and purified in a previous work.[7] Deionized and twice-distilled water was used. The PVA film used for viscoelasticity measurements was obtained by casting from an ethanol solution. Electronic spectra were measured with a Shimadzu spectrophotometer Model MPS-5000. Mechanical properties were measured on a Toyo-Boldwin viscoelastometer Model DDV-II-C.

Results and Discussion

Spectral Features. A difference spectrum between an squeous solution of Cu-chln plus PVA and that of Cu-chln alone had a maximum at 652 nm (Fig. 1). To estimate the equilibrium constant of the complex, the Benesi-Hildebrand plot,[8] the plot of the reciprocal height of the difference spectrum at 652 nm against the reciprocal concentration of added PVA, was performed and the data were analayzed according to the equation:

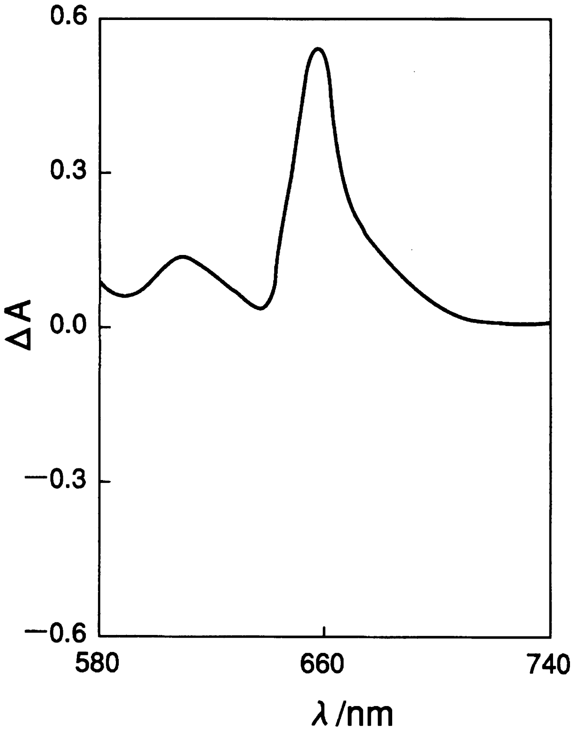

Fig. 1. Difference spectrum between an aqueous solution of copper chlorophyllin plus PVA and that of copper chlorophyllin alone. Sample: [Cu-chln], 10^{-4} $\text{mol}\,\text{dm}^{-3}$ and [PVA], 0.1 $\text{mol}\,\text{dm}^{-3}$; Reference: [Cu-chln], 10^{-4} $\text{mol}\,\text{dm}^{-3}$.

$$\frac{C_\text{t}}{OD_\text{c}} = \frac{1}{K\varepsilon_\text{b}}\frac{1}{C_\text{A}} + \frac{1}{\varepsilon_\text{b}}, \tag{1}$$

here C_t is the total Cu-chln concentration, OD_c the height of the difference spectrum, K the equilibrium constant, ε_b the molecular extinction coefficient, and C_A is the concentration of PVA added.

The Benesi-Hildebrand plots for Cu-chln in the presence of UMR-20H and UMR-20M are shown in Fig. 2. The data points are well on straight lines, and the equilibrium constants are estimated to be 833 and 1820 $\text{mol}^{-1}\,\text{dm}^3$ from the slopes. Namely the equilibrium constant is larger for the Cu-chln/UMR-20M system than the Cu-chln/UMR-20H system. Since PVA of lower degree of saponification has more acetyl groups, Cu-chln apparently interacts more strongly with the acetyl group than with the hydroxyl group. In a separate experiment, the equilibrium constant between Cu-chln and poly(vinyl acetate) was found to be 2640 mol^{-1} dm^3 in acetone.

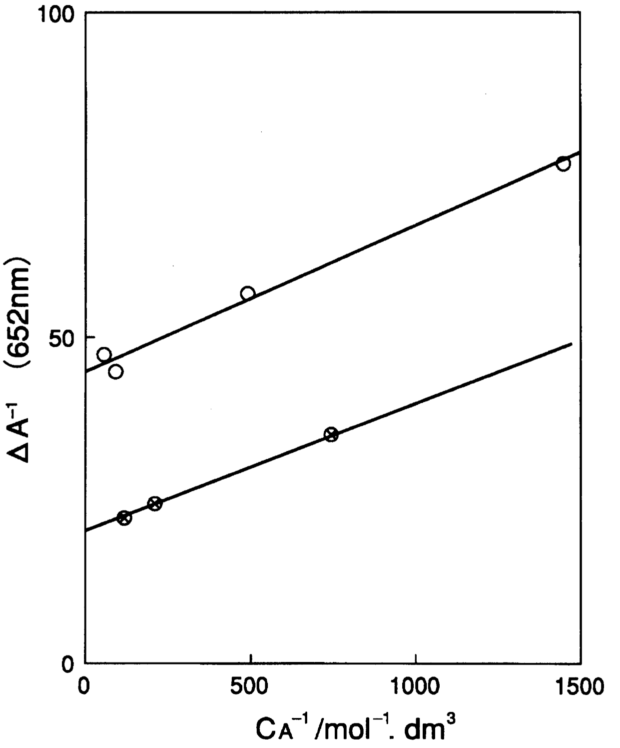

Fig. 2. Beneshi-Hildebrand plots between Cu-chln and PVA (UMR-20H and UMR-20M) -⊗-; UMR-20H, degree of saponification: 81.7%, -o-; UMR-20M, degree of saponification: 63.8%, [Cu-chln]=10^{-4} mol dm $^{-3}$.

These data were subjected to the Scatchard plot.[9] We can estimate the equilibrium constant K and the binding number n by analyzing the plot with the equation:

$$\frac{C_{\mathrm{chlb}}/C_{\mathrm{A}}}{C_{\mathrm{chlf}}} = -K\left(\frac{C_{\mathrm{chlb}}}{C_{\mathrm{A}}} - n\right), \tag{2}$$

here C_{chlb} and C_{chlf} are the concentrations of bound and free Cu-chln, respectively, and C_{A} the concentration of PVA repetition units. The Scatchard plots were performed for a Cu-chln concentration fixed at 10^{-4} mol dm^{-3} and varying the concentration of PVA repetition units from 6.0×10^{-4} to 0.14 mol dm^{-3}, and the results are given in Figs. 3 and 4. The plot in Fig. 3 is rectilinear while that in Fig. 4 is superlinear. The latter indicates that the larger the Cu-chln concentration per polymer site, the more Cu-chln binds to the site. This suggests the occurrence of a cooperative binding as reported for the interaction between Mn^{2+} and t-RNA.[10]

Inamura et al.[11] found that the bond between chlorophyll and a polymer was not broken by dilution down to at least 10^{-4}% and the complex between chlorophyll and the polymer existed stably. Uehara et al.[12] measured the electronic spectra of chlorophyll a bound to water soluble polymers and discriminated several components peaking at 650 to 800 nm, to conclude the existence of energy transformation from fluorescent spectra.

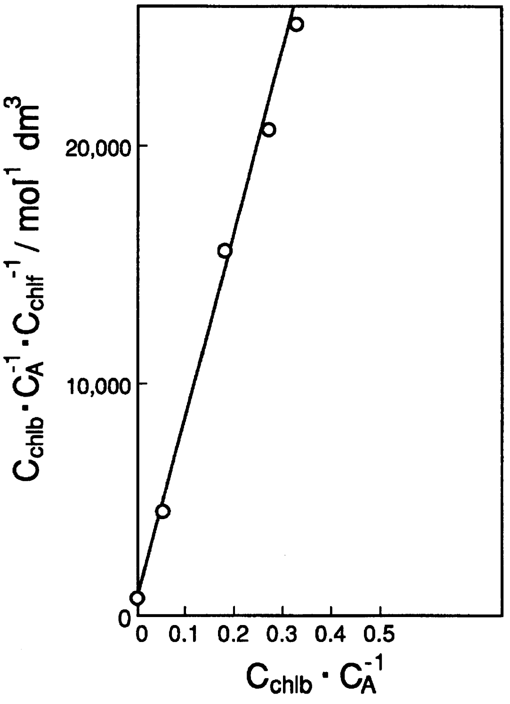

Fig. 3. Scatchard plot between Cu-chln and PVA (UMR-20H).

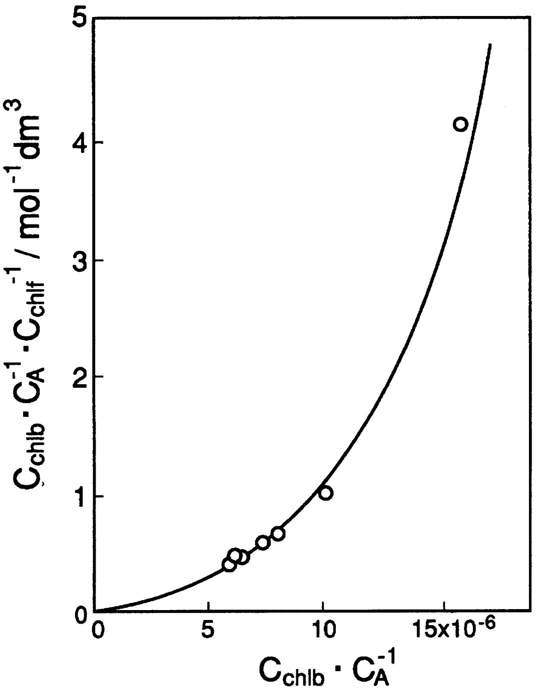

Fig. 4. Scatchard plot between Cu-chln and PVA (UMR-20M).

That is to say, Cu-chln bound to poly(vinyl alcohol) does not always exist in a single state but exist in various states. As is shown in Cu-chln–PVA complex, the existence of cooperative binding is assumed in a chlorophyll-protein complex. There may exist an interaction

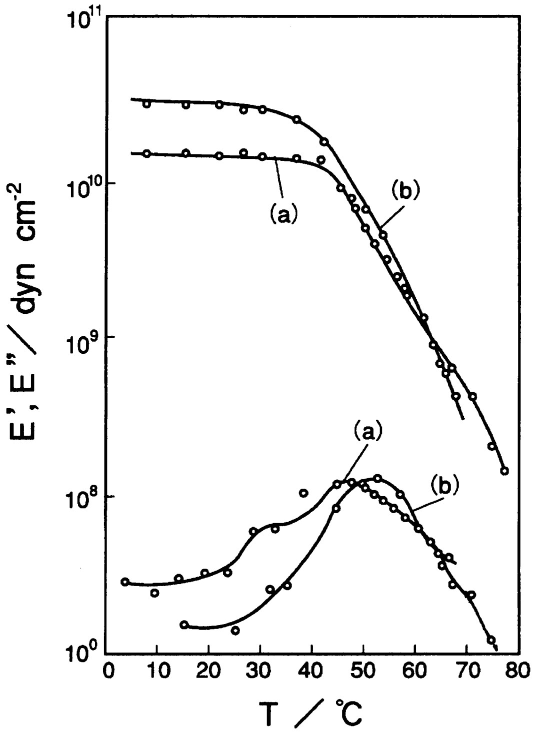

Fig. 5. Viscoelastisity of PVA measured at 11 Hz. (a) PVA (UMR-20L) alone, (b) PVA + 1 mol% Cu-chln, Frequency: 11 Hz.

among the sites in the protein, and the more the chlorophyll binds to protein, the larger the binding strength. This is in line with the observation that chlorophyll can exist in concentrations as high as 1 mol dm^{-3}. Evidence for the high-polymerization of Cu-chln was obtained by another experiment (unpublished results).

Mechanical Properties. The viscoelasticity of the PVA film was measured in the absence and presence of Cu-chln, and the resuls for UMR-20L are shown in Fig. 5. We chose UMR-20L in this experiment for resons of hygroscopicity of the polymer film. E$'$ denotes the loss modulus and E$''$ the storage modulus. As is seen the curve shifted to higher temperatures in the presence of Cu-chln. This suggests that the chains of PVA are bridged in the presence of Cu-chln, because the latter coordinates to the OH group in PVA. The loss modulus (E$'$) of PVA containing Cu-chln is 1.5 times larger than that of PVA alone. This phenomenon again indicates that the side chains are bridged each other and produces net-working polymer. As an application, the UMR-20L-Cu-chln system may give a water-proof and strong cooking film.

References

1) C. S. French, J. S. Brown, and M. C. Laurence, *Plant Physiol.*, **45**, 421(1972).

2) I. Inamura, K. Toki, T. Araki, and H. Ochiai, *Chem. Lett.*, **1980**, 1481.

3) K. Enmanji, *Nippon Kagaku Kaishi*, **1977**, 438.

4) K. Enmanji and S. Etho, *Nippon Kagaku Kaishi*, **1983**, 1231.

5) K. Enmanji, *Nippon Kagaku Kaishi*, **1983**, 1535.

6) K. Enmanji, *Nippon Kagaku Kaishi*, **1986**, 1216.

7) K. Enmanji, *Nippon Kagaku Kaishi*, **1978**, 1317.

8) H. A. Beneshi and J. H. Hildebrand, *J. Am. Chem. Soc.*, **91**, 2703 (1949).

9) G. Scatchard and E. S. Black, *J. Phys. Colloid Chem.*, **53**, 88 (1949).

10) A. Danchin and M. Guron, *Eur. J. Biochem.*, **16**, 532 (1970).

11) I. Inamura, M. Ochiai, K. Toki, S. Watanabe, S. Hikino, and Y. Araki, *Photochem. Photobiol.*, **38**, 37 (1983).

12) K. Uehara, M. Mimuro, Y. Fujita, and M. Tanaka, *Photochem. Photobiol.*, **48**, 725 (1988).