CHEMISTRY LETTERS, pp.291-294, 1976. Published by the Chemical Society of Japan

INTERACTION BETWEEN DYES AND POLYELECTROLYTES III. STRUCTURAL EFFECT OF POLYANIONS ON THE FORMATION OF AGGREGATED METHYLENE BLUE

Masamitsu SHIRAI, Tatsuki NAGATSUKA, and Makoto TANAKA
Department of Applied Chemistry, Faculty of Engineering, University of Osaka Prefecture, Sakai, Osaka 591

Effects of the structures of polyvinylsulfate and its homologues on the aggregation of methylene blue were studied in aqueous solution. Important factors were the number of anionic site per polymer molecule and the flexibility of polymer chain.

An obvious color change is observed when cationic dyes are bound to polyanions. This phenomenon is well known as metachromasy.[1] Methylene blue(Mb) is a representative cationic dye showing metachromasy.[2] The metachromatic behavior still remains unclear because of its complexity. In the present communication we wish to report that the metachromatic behavior of Mb in the presence of polyvinylsulfate or its homologues is very susceptible to the constitution of polyanions. Studies on the binding of Mb to macromolecules[3] and on the reactions(redox and photochemical) of the bound Mb[4] are of great interest in relation to the functions of coenzyme in oxido-reductase and chlorophyll in chloroplast lamella.

Table I. Characteristics of Polyanions used

polyanion	potassium polyvinylsulfate							potassium polyallylsulfate	potassium polymethallylsulfate
	1	2[a]	3	4	5	6	7	8	9
$\overline{Pn}$[b]	1500	1500	1500	1500	1500	500	150	250	410
α[c]	100	50	50	30	12	93	100	96	82

a) unsulfated -OH groups(2 mole%) are allowed to react with formaldehyde.
b) degree of polymerization. c) degree of sulfation(mole%).

The polyanions were prepared by the sulfation of the corresponding polyalcohols with chlorosulfonic acid in pyridine and listed in Table I. The degree of sulfation was determined from elemental analysis. Absorption spectra were taken on a Shimadzu UV-200 recording spectrophotometer at a given temperature. Aqueous sample solutions were always equilibrated for 10 minutes before measurements of the absorption spectra.

Figure 1 shows the absorption spectra of Mb in the presence of different amounts of potassium polyvinylsulfate(1 in Table I). The absorption maximum of Mb at 664nm (monomeric Mb) decreased and a new absorption band(metachromasy band) appeared near 550nm on addition of the polyanion. This spectral changes are similar to those occurring upon increasing the concentration of an aqueous Mb solution.5) Addition of excessive polyanion, as well as addition of ethanol or urea, caused the disappearance of the metachromasy band and the restoration of the absorption band at 664nm. These facts suggest that the absorption band near 550nm arises from the aggregation of Mb on the polyanion chain.6-8) Similar spectral changes were observed on addition of other polyanions.

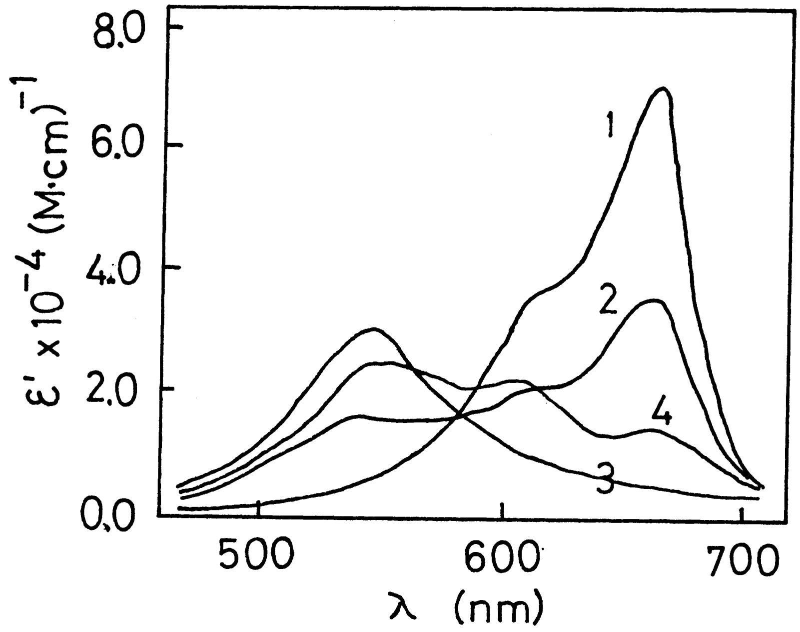

Figure 1. Absorption spectra of Mb in the presence of different amounts of potassium polyvinylsulfate(1):
1) 0,
2) 1.3×10^{-5} equiv./l,
3) 1.3×10^{-4} equiv./l,
4) 1.3×10^{-2} equiv./l.
[Mb]$=1.1 \times 10^{-5}$M.

The metachromatic behavior was markedly affected by the value of P/D([anionic site of polyanion]/[Mb]). The apparent molar extinction coefficients(ε') of Mb at 664nm in the presence of polyanions were plotted against P/D values on a logarithmic scale at 17°C. The P/D values were varied by adjusting the polymer concentrations at a fixed Mb concentration. A representative result was shown in Figure 2. On addition of 1, ε' decreased with increasing concentration of 1 and showed a minimum (ε'_{min}) at the P/D value of about 2. After holding an approximately constant value over the range of P/D between 2-56, ε' increased again to regain its original value ($7.1\times10^{4}M^{-1}cm^{-1}$) at P/D value of about 56. Similar relations between ε' and P/D value were observed for other polyanions.

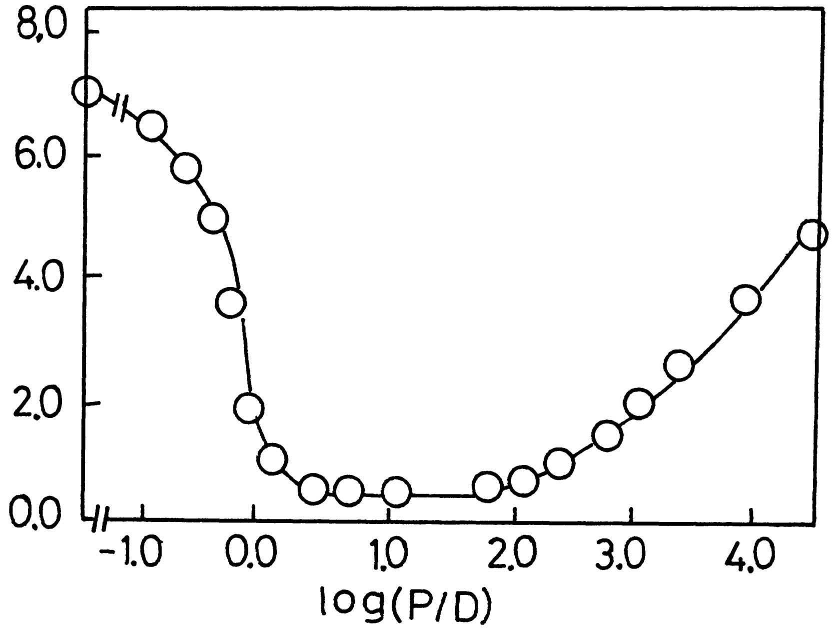

Figure 2. Effect of P/D values on the apparent molar extinction coefficient(ε') at 664nm. $[Mb]=1.1\times10^{-5}M$, at 17°C.

Numerical values of ε'_{min} and $(P/D)_{min}$ which means the range of P/D value over which ε' holds a minimum value are summarized in Table II, along with λ_m which is the wavelength of metachromasy band. As shown in Figure 1, a rough isosbestic point appeared below the P/D values where ε' began to increase. A similar change in spectrum was observed for all polyanions used. This fact means that in the P/D region a decrease in ε' should correspond to an increase in the concentration of aggregated Mb. As seen in Table II, the polyanion showing a small ε'_{min} gives a wide range of $(P/D)_{min}$ and a short λ_m. Thus it is reasonable to consider that the polyanion which shows a small ε'_{min}, a wide range of $(P/D)_{min}$, and a short λ_m has the high efficiency of aggregation of Mb. In potassium polyvinylsulfate series it can be seen that among the

Table II. Structural effect of polyanions on the formation of aggregated Mb[a)]

polyanion	1	2	3	4	5	6	7	8	9
$(P/D)_{min}$	2-56	2-13	2-16	3-17	4-11	2-22	2-22	2-6	1-7
$\varepsilon'_{min}\times10^{-3}$ ($M^{-1}cm^{-1}$)	3.8	6.8	3.8	7.0	13.5	4.7	4.4	5.3	11.3
λ_m (nm)[b)]	545	548	548	553	560	548	550	550	555

a) numerical values are obtained at 17°C. b) wavelength of metachromasy band where ε'_{min} is given polyanions having similar degree of sulfation(α), the efficiency of aggregation of Mb decreases with decrease in degree of polymerization($\overline{Pn}$)($1 > 6 \simeq 7$) and that among the polyanions having the same $\overline{Pn}$, the efficiency of aggregation of Mb decreases with decrease in α($1 \simeq 3 > 4 > 5$). This seems to suggest that the number of anionic site on one polymer molecule is an important factor for the aggregation of Mb. The structural effect of monomer unit was compared among 6, 8, and 9. Although these polyanions somewhat differ from each other in their $\overline{Pn}$ and α, such differences seem to be insignificant, considering the similar efficiency between 1(α=100) and 3(α=50) and between 6($\overline{Pn}$=500) and 7($\overline{Pn}$=150). It was found that the efficiency of aggregation of Mb decreased in the order $6 \simeq 8 > 9$, meaning that both the methylene unit inserted between anionic site and main chain and the methyl group attached to main chain resulted in a decrease of the efficiency of aggregation of Mb and the effect of the latter was more significant. Thus, it is ascertained that the efficiency of polyanions for aggregation of Mb is appreciably affected by $\overline{Pn}$, α, and structure of monomer unit. It seems undoubted that the aggregation of Mb by addition of polyanions takes place *via* electrostatic binding of Mb to polyanions followed by aggregation of Mb bound on the same polymer molecule. If any effective conformational change of polymer chain does not contribute to the aggregation of Mb, $(P/D)_{min}$ should be unity for all polyanions studied. Actually, however, $(P/D)_{min}$ took the values more than 2, showing certain ranges as shown in Table II. The importance of flexibility of polyanions for aggregation of Mb is supported by the fact that the efficiency of aggregation of Mb for 3 is larger than that for 2 whose unsulfated -OH groups(2 mole%) were treated with formaldehyde. The effective conformational change must be more advantageous for more flexible polymers. The fact that the efficiency of aggregation of Mb for 9 is less than those for 6 and 8 may be due to less flexibility of the former. No ability of potassium polystyrenesulfonate[3)] for the aggregation of Mb might be ascribed to the structural effect.

Details should be investigated.

REFERENCES

1) M. Miura and Y. Kubota, Bull. Chem. Soc. Japan, 40, 466(1967).
K. Yamaoka, M. Takatsuki, K. Yaguchi, and M. Miura, ibid., 47, 611(1974).
V. Vitagliano and L. Costantino, J. Phys. Chem., 74, 197(1970).
B. Simionescu and G. J. Smets, Makromol. Chem., Suppl. 1, 249(1975).
2) B. Carroll and H. C. Cheung, J. Phys. Chem., 66, 2585(1962).
3) M. Shirai, T. Nagatsuka, and M. Tanaka, in contribution.
4) M. Shirai and M. Tanaka, J. Polym. Sci., A-1 13, (1975) in press.
5) M. Schubert and A. Levine, J. Amer. Chem. Soc., 77, 4197(1955).
6) D. F. Bradley and M. K. Wolf, Proc. Natl. Acad. Sci. USA, 45, 944(1959).
7) M. K. Pal and M. Chaudhuri, Makromol. Chem., 133, 151(1970).
8) P. Mukerjee and A. K. Ghosh, J. Phys. Chem., 67, 193(1963).

(Received December 24, 1975)