CHEMISTRY LETTERS, pp. 583-586, 1982.

THE J BAND OF THE MALACHITE GREEN-POLY(α-L-GLUTAMIC ACID) COMPLEX: THE CONDITIONS FOR ITS APPEARANCE IN AQUEOUS SOLUTIONS

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The optimum conditions for the appearance of the J band of Malachite Green bound to poly(α-L-glutamic acid) are established by the method of absorption spectroscopy. The J band in the visible spectra depends very critically on such factors as pH, aging, mixing ratio, salt concentration, and, above all, the polymer conformation.

It was first reported in a previous paper that Malachite Green (MG) can bind to poly(α-L-glutamic acid), $(\mathrm{Glu})_n$, showing a very narrow and intense absorption band at 684 nm on the long-wavelength side of the principal band at 620 nm.$^{1)}$ This spectral feature has been termed the J band, since it is similar to the well-known phenomenon originally observed by Jelley for some isocyanine dyes without host polymers.$^{2)}$ It was also noted that the J band of MG is sensitive to the conformation of $(\mathrm{Glu})_n$, which undergoes a pH-induced helix-coil transition, and to the chemical structure of triphenylmethane dyes, among which Parafuchsine and Crystal Violet showed no sharp absorption band.$^{1,3)}$ We can now present in detail the conditions for the appearance of the J band in the absorption spectrum of the MG-$(\mathrm{Glu})_n$ complex in aqueous solutions. The pH, the mixing ratio of polymer residue-to-dye (P/D), the salt concentration, and the aging of sample solutions were all found to be the factors which affect the unusual optical behavior of MG bound to $(\mathrm{Glu})_n$.

The MG sample (Chroma) was assayed for the oxalate anion,$^{4)}$ and purified from the carbinol solely as the chloride form. The sodium salt of $(\mathrm{Glu})_n$$^{3)}$ was dialyzed against HCl of appropriate concentrations for 5 days at 25 °C, in order to remove both sodium and chloride ions which are produced in solution as the ionized side-chain carboxylate groups become protonated. The pH of the $(\mathrm{Glu})_n$ solution was usually lowered to 4.4–4.7 by the dialysis. To this $(\mathrm{Glu})_n$ solution, the pH-adjusted MG solution was added under gentle stir. A part of this mixture was used for the pH determination. Absorption spectra were measured at 25 °C on a Hitachi Model EPS-3T recording spectrophotometer. The slit width was carefully controlled, so that the observed peak intensity of the sharp J band was free from any artifacts.

The effect of pH on the absorption spectra of the MG-$(\mathrm{Glu})_n$ complex is shown in Fig. 1. The changes in the 620-nm and 425-nm absorption bands indicate that MG binds to $(\mathrm{Glu})_n$ in the pH range 6–4, but the J band appears only when the pH is lower than 5.6. Hence, the origin of the J band and the formation of the complex are not necessarily parallel to each other. The changes in the molar absorption coefficient, ε, show that the J band is limited to a very narrow pH range and its intensity becomes higher than that of the principal band at pH less than 5.0. The half-intensity band width, where the peak intensity decreases by one-half, is only 450 cm^{-1} for the J band, while it is 1650 cm^{-1} for the 620-nm band (pH = 4.72), indicative of the narrowness of the J band. The remarkable effect of the aging of the MG-(Glu)$_n$ system on the J band is shown in Fig. 2. The full development of the J band clearly requires a sufficient period of time—a surprising fact discovered in this work. The position of the J band remains unshifted with time, but the intensity increases rapidly within 12 hours. The gain of the J-band intensity is associated with the loss of the 620-nm band intensity. The time dependence of the full development of the J band becomes more pronounced, as the pH of the MG-(Glu)$_n$ solution decreases. The change of ε with time could not be expressed by the first or the second order reaction rate with respect to the MG concentration.

The P/D dependence of the absorption spectra and the intensity of the 684-nm and 620-nm bands are shown in Fig. 3. The appearance of the J band clearly depends on values of P/D. It is most favored in a narrow P/D range of 20–100 at the pH, where the (Glu)$_n$ backbone is in the helical conformation, while the principal band shows a different P/D dependence. Figure 3b shows the effects of sodium acetate buffer and of the MG concentration which was varied between 0.8 x 10^{-5} and 5 x 10^{-5} M. The optimum MG concentration for the J band is ca. 2 x 10^{-5} M. The decrease in values of ε at higher P/D, in each case, is probably due to the dissociation of MG

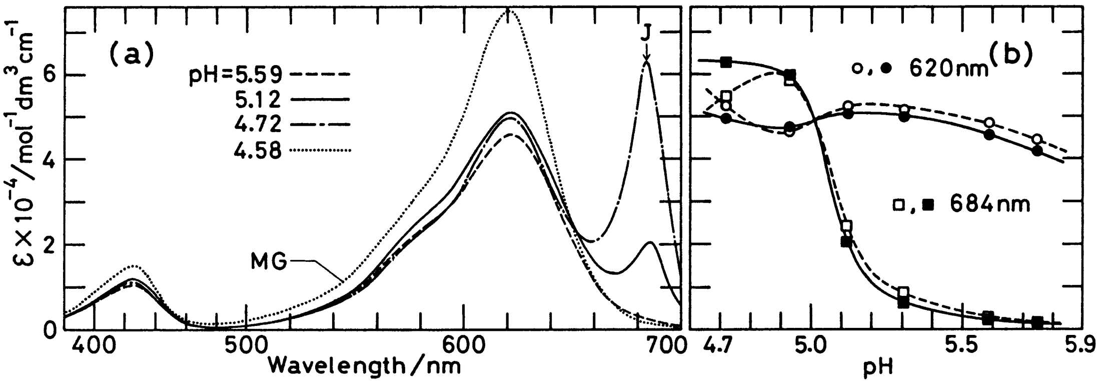

Fig. 1. The effect of pH on the absorption spectra of MG-(Glu)$_n$ solutions aged for 10 days in (a) and 10 days (●,■) and 3 days (○,□) in (b). The J band is at 684 nm and the principal band at 620 nm. P/D=34, [Glu]=0.8 mM, and [Buffer]=80 μM.

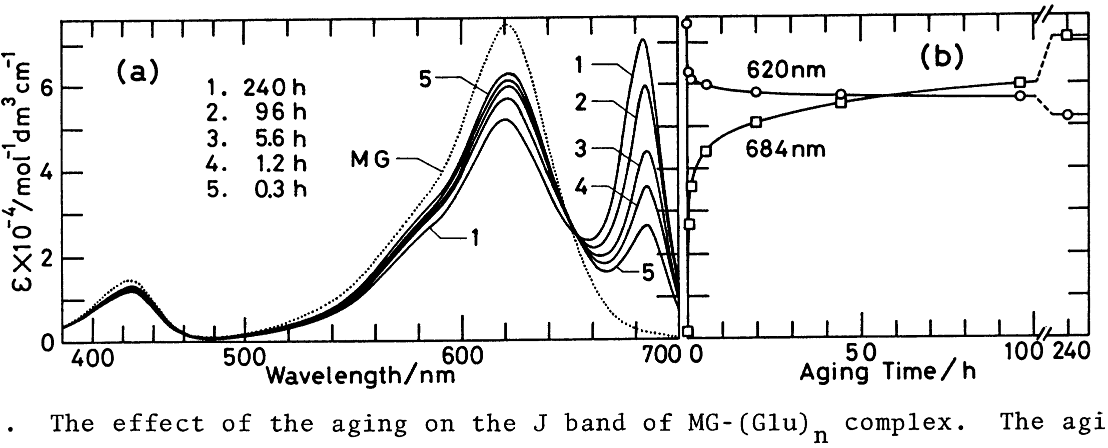

Fig. 2. The effect of the aging on the J band of MG-(Glu)$_n$ complex. The aging time in hours is given in (a). pH=4.43 and other conditions are the same as in Fig. 1.

from the polymer site. The amount of MG actually bound to (Glu)$_n$ was estimated by equilibrium dialysis.[5] The results in terms of the binding fraction of MG, f_b, are as follows:[5] 0.8 (no added NaCl), 0.70 (in 0.4 mM NaCl, 1 M = 1 mole/dm^3), and 0.66 (in 20 mM NaCl) for the MG-(Glu)$_n$ system at pH 4.4 and P/D 34. The very pronounced effect on the development of the J band is given by the concentration of sodium ions added to the MG-(Glu)$_n$ solutions at low pH's, as is shown in Fig. 4. The J-band intensity diminishes and eventually disappears completely with the increase in NaCl concentrations, while the 620-nm band intensity gradually approaches the value of MG without (Glu)$_n$. The conditions adopted here are most favorable for the J band to appear (Figs. 1, 2, and 3); therefore, the presence of neutral salts such as NaCl and KCl is very critical and the excess counterions must be removed from the MG-(Glu)$_n$ solution in order to observe the fully developed J band. This is the reason why the (Glu)$_n$ solution was subjected to exhaustive dialysis before mixing with MG. Although the excess neutral-salt ions tend to dissociate MG from (Glu)$_n$, the results of the equilibrium dialysis indicate that nearly 70% of MG molecules remain bound in the presence of 20 mM NaCl. Therefore, the dissociation of MG is not parallel to the disappearance of the J band.

The correlation between the conformation of the MG-(Glu)$_n$ complex and the

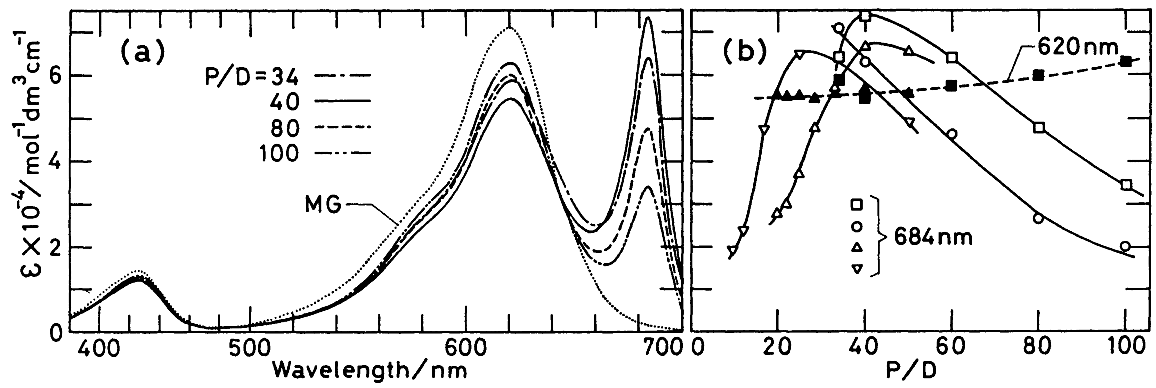

Fig. 3. The P/D dependence of the absorption spectra of MG-(Glu)$_n$ solutions aged for 10 days. (□,■) [Glu]=1.0 mM, pH=4.8 in (a) and (b). (○) [Glu]=0.8 mM, pH=4.4, [Buffer]=80 μM, (△,▲) [Glu]=1.0 mM, pH=4.6, and (▽) [Glu]=0.5 mM, pH=4.6 in (b).

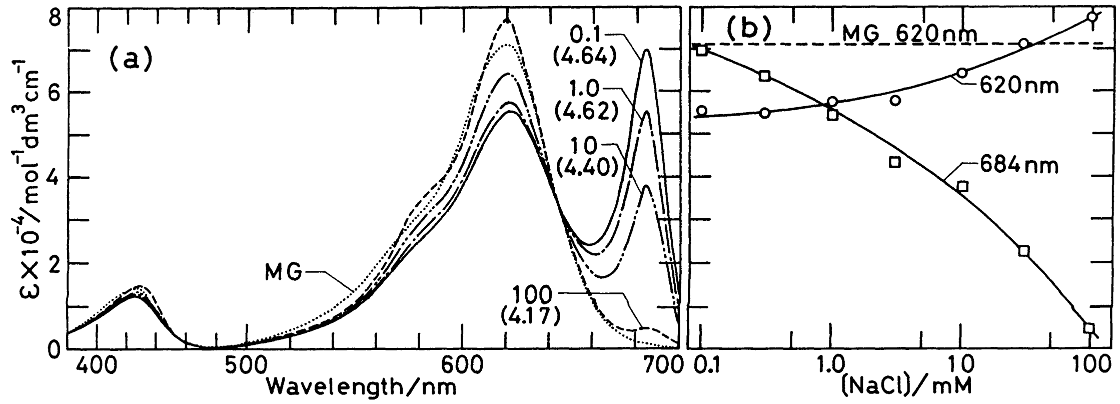

Fig. 4. The dependence of the J-band intensity on the concentration of NaCl added to MG-(Glu)$_n$ solutions. The solutions are aged for 10 days. P/D=34 and [Glu]=0.8 mM. Numerals in (a) denote the NaCl concentration in mM and the pH in parentheses.

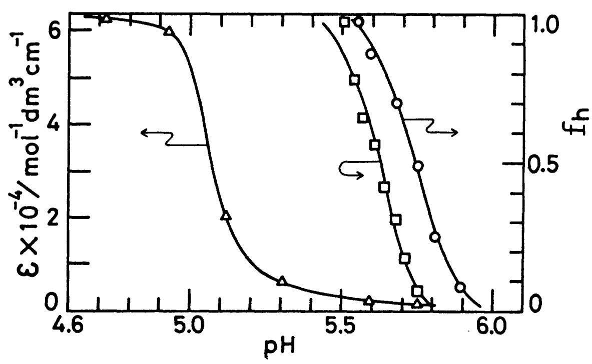

Fig. 5. The pH-induced conformational change and the appearance of the J band of the MG-$(Glu)_n$ complex at 684 nm (△). P/D=34 and [Glu]=0.8 mM (cf. Fig. 1). The fraction of helix, f_h, is (○) for MG-$(Glu)_n$ and (□) for $(Glu)_n$ only. The weight-average degree of polymerization of $(Glu)_n$ is ca. 5×10^2.

appearance of the J band is shown in Fig. 5, where the fraction of helix, f_h, was plotted against pH. Values of f_h were obtained by the method of Nagasawa and Holtzer[6] from the potentiometric titration data of $(Glu)_n$ in the presence and the absence of MG. The pH-induced helix-coil transition of the $(Glu)_n$ backbone occurs in a very narrow pH range of 5.5–6.0, the midpoint (f_h = 0.5) being 5.63 for $(Glu)_n$ and 5.75 for MG-$(Glu)_n$. Surprisingly, the J band is still very weak even at a low pH of 5.5, at which the helical formation is almost completed. It is in the so-called aggregation region,[5-8] but not near the onset of the helix region, that the J band is fully developed. This is a new and important finding to be noted. Since the aggregation of $(Glu)_n$ helices probably involves the rearrangement of the side-chain carboxylate groups to which MG is bound, the electronic configuration of the bound MG must be favorable for the sharp J band, for which the proper mechanism is yet to be proposed in future. To elucidate the origin of the J band, we are now investigating the orientation of the bound MG chromophores relative to the helical backbone of $(Glu)_n$ in the visible wavelength region by the electric dichroism method,[9] the reversibility of the J band and the conformation with temperature, and the possibility that the J band is generally associated with other diaminotriphenylmethane dyes.

In conclusion, the detailed experimental conditions were clarified for the first time for the appearance of the J band in the absorption spectra of MG-$(Glu)_n$ complexes. This peculiar optical behavior is confined to the P/D range 20–100, the pH range 4.5–5.5, and the MG concentration of about 2×10^{-5} M in aqueous solutions which contain the least amount of the free counterions, Na^+, competing with MG cations for the ionized side-chain carboxylate group.

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(Received February 10, 1982)