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EXTRINSIC COTTON EFFECTS ASSOCIATED WITH THE J- AND γ-BAND OF TRIPHENYLMETHANE DYES BOUND TO POLY-α,L-GLUTAMIC ACID

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Spectroscopically "metachromatic" crystal violet shows a characteristic γ-band and, by comparison, "normal" malachite green gives rise to a sharp J-band, when each dye is bound to poly-α,L-glutamic acid in helical conformation. These absorption bands are associated with induced Cotton effects of very high magnitude hitherto not observed for acridine dyes.

Although symmetrically substituted acridine dyes such as acridine orange and proflavine all belong to a symmetry group of C_{2v} and hence are optically inactive, they become optically active and give rise to extrinsic Cotton effects in the visible region of their absorption bands when bound to optically active biopolymers such as deoxyribonucleic acid (DNA) and poly-α,L-glutamic acid (PLGA).1) To further understand the mechanism of this induced optical activity of the dye-biopolymer complex, some work is now being undertaken with special emphasis on the effect of molecular symmetry and chemical structure of cationic dyes.2) This report presents the preliminary result of induced optical rotatory dispersion (ORD) and optical absorption of two representative triphenylmethane dyes--malachite green (MG), which lacks a threefold symmetry axis and not metachromatic, and crystal violet (CV), which possesses the symmetry axis and frequently metachromatic--in the presence of PLGA in aqueous media.

A PLGA-MG complex in aqueous solution at a pH of about 4.5, where the polymer alone is in a helical conformation, shows a remarkable absorption spectrum with a very narrow band centered at 684 nm in the longer wavelength side of the principal band at 620 nm, as shown in Figure 1. The intensity of this new band, which is similar to the one commonly known as J-band,3,4) depends intricately on such conditions as pH, temperature, salt concentration, dilution, the ratio of polymer residues to dye (P/D),1) and standing time, all of which indicate that the polymer conformation is a critical factor.5) The appearance of such J-bands in the presence of various polyelectrolytes has previously been reported for the micellar pseudoisocyanine[6)] and methylthiocarbocyanines,[7,8)] both of which are strongly metachromatic. Stryer and Blout showed that the J-band was optically active in the presence of PLGA and of its isomer PDGA.[6)] Scheibe, et al. also observed circular dichroism (CD) for the J-band of heparin complexes.[8)]

In contrast with those dyes, however, MG is "normal" spectroscopically, that is, Beer's law is obeyed in aqueous solutions, and has been reported to be either influenced little or only hypochromic in the presence of DNA,[2,9)] agar,[10)] polystyrene sulfonate,[9)] polyphosphate,[11)] gelatin and PLGA in random-coil conformation.[5)] This is the first example that a non-metachromatic dye can exhibit a sharp J-band which is associated with an extrinsic Cotton effect of exceedingly large magnitude, as the molar rotation [R] for this band only on the right ordinate indicates (Fig. 1). This Cotton effect appears to be multiple, when its positions

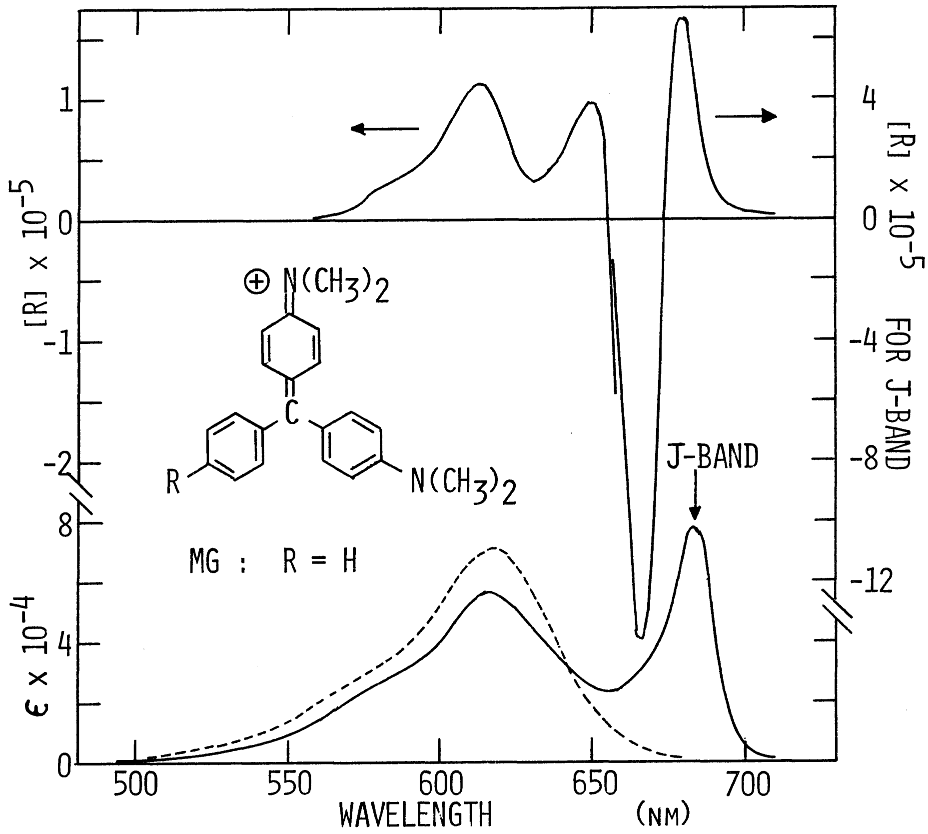

Fig. 1. The ORD (upper) and absorption spectra (lower) of malachite green in the presence of poly-α,L-glutamic acid.
———PLGA-MG complex at P/D=167 and pH=4.5 to 4.6; -------MG alone for comparison. [MG]=4.37 x 10^{-5} M. The definition of molar rotation [R] and molar extinction coefficient ε is the same as in Ref. 1. The ORD was measured with a Cary Model 60 spectropolarimeter at 25-27°C. All dyes as chloride salts (Chroma Gesellschaft, Schmid & Co.) were used without further purification.

and shape are taken under consideration. The other three ordinary absorption bands of bound MG at 620, 425 and 317 nm also become optically active.

The absorption band of crystal violet with a strong maximum at 592 nm with a shoulder at about 550 nm splits into three peaks in the presence of PLGA in aqueous solutions. As shown in Figure 2, a distinct maximum at about 505 nm in the wavelength shorter than the principal band of CV is characteristic of PLGA-CV complexes. This new band corresponds to the γ-band according to Michaelis's nomenclature for metachromasy;[12] the α-band at 590 nm and the β-band at about 550 nm. Contrary to the property of the J-band of MG, the intensity of this γ-band is rather insensitive to the pH of PLGA-CV solutions and it appears, over a wide range of P/D, in both acid and neutral aqueous media where the polymer alone is either helical or random-coil. This leads to the conclusion that the γ-band of the bound CV is not conformation-dependent. In fact, the appearance of such γ-band at about 505 nm is known for the complexes with polyphosphate[11] and polyacrylate.[13] In any case, however, CV shows no J-band in the presence of aforementioned polyelectrolytes.

The α-, β-, and γ-band of CV all become optically active when it is bound to PLGA in either acid or neutral medium. This indicates that the comformation of PLGA

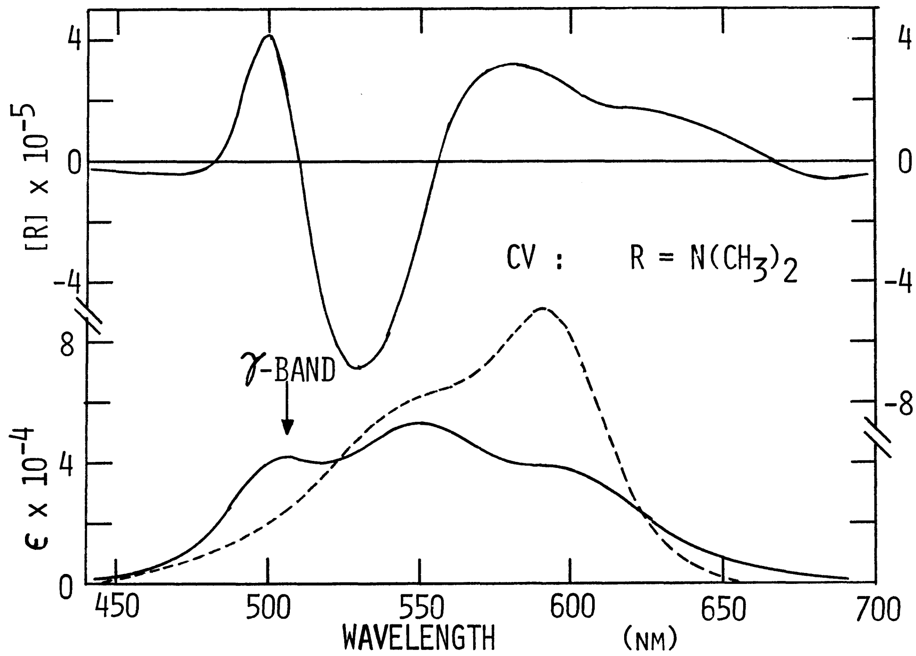

Fig. 2. The ORD (upper) and absorption spectra (lower) of crystal violet in the presence of poly-α,L-glutamic acid.
———PLGA-CV complex at P/D=270 and pH=4.5 to 4.6; -------CV alone for comparison. [CV]=2.69 x 10^{-5} M.

is not a necessary condition for induced optical activity associated with the γ-band. While both α- and β-band are known to appear in the presence of DNA,[9] these bands are also found to be optically active and to show extrinsic Cotton effects which are again little dependent on the degree of heat-denaturation of DNA.[2,5]

Parafuchsin, which has the same symmetry and chemical structure as CV except for three unsubstituted amino groups but is not metachromatic, also shows the extrinsic Cotton effect in the visible region, in spite of the fact that the absorption spectrum in the presence of PLGA is only hypochromic and reveals neither γ-band nor J-band.[5] Whether or not they either possess a threefold symmetry axis or are metachromatic, cationic triphenylmethane dyes can exhibit the extrinsic Cotton effect when bound to PLGA. Moreover, CV and MG each show their additional chracteristics; the conformation-insensitive γ-band and the conformation-sensitive J-band. It thus appears to be possible to utilize those syes as a coarse or fine structural probe, by the method of ORD or CD, to study detailed conformations of PLGA. In this connection, it should be interesting to extend the present study to acidic homo- and heteropolypeptides as well as proteins containing acidic amino acid residues, because the effective means of differentiating nucleic acids from proteins on cellular level should always be sought out.

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