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Irreversible Conformational Change of the Complex of Poly(L-lysine) with Methyl Orange upon Irradiation of Light

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Synopsis. The effect of light on the chiral complex of poly(L-lysine) (PLL) with Methyl Orange (MO) was studied by means of absorption and circular dichroism (CD) measurements. Upon irradiation, the magnitude of the induced CD of the PLL–MO complex decreased together with a decrease in dimeric dye species. The change was irreversible under dark or visible-light conditions.

Reversible conformational changes induced by light in azobenzene-containing polypeptides in organic solvents have been studied by different groups.[1–3] Conformational changes induced by light in water-soluble poly(L-glutamic acid) (PLG) with photochromic side chains have also been studied by Pieroni *et al.*[4,5] Though a *trans* to *cis* photoconversion in an aqueous solution was lower than in an organic solvent, the photochemical reactions in both solvents were entirely reversible.[4,5]

We have investigated the induced CD of cationic polypeptides and azo dyes[6–9] and wish to report here the effect of light irradiation on the PLL–MO complex in water.

Experimental

The PLL HBr sample used had a polymerization degree of 3260. 4-(4-Dimethylaminophenylazo)benzenesulfonic acid sodium salt (Methyl Orange, MO) was a guaranteed reagent (Wako). A MO solution was prepared in red light and kept in the dark. The final concentrations of MO were 2×10^{-5} mol dm^{-3}, and the ratio of lysyl residues to dye molecules (R/D) was 5. The absorption and CD spectra were measured with a Jasco UVIDEC-1 and a CD J-40A spectrometer. All the following data were reduced to the molar basis of the total dye concentration or the lysyl residue concentration, and were expressed in terms of the ellipticity $[\theta]$ (degree cm^2 dmol^{-1}) and molar extinction coefficient ε (dm^3 mol^{-1} cm^{-1}).

Irradiations of the sample solutions were carried out with a mercury lamp (400 W) filtered with narrow-band interference filters (UV-D36A for 360 nm and B-46 for 460 nm) from Toshiba. The light intensity was measured by actinometry.[10]

Results and Discussion

The MO showed a single visible absorption band at 467 nm with ε_{467}=25600 in water (at a neutral pH). Upon complexation with PLL, an additional blue-shifted band was observed at 367 nm due to the dimeric dye species,[11,12] exhibiting an induced CD. The magnitudes of the induced CD of the PLL HBr–MO complex were −128000 (371 nm) and 129000 (359 nm) at pH 6 and R/D=5. Figure 1 shows the time dependence of the absorption spectra of the PLL HBr–MO complex upon irradiation at 360 nm (2.7×10^{19} photons cm^{-2} s^{-1}).[10] The intensity of the absorption band at 367 nm decreased and a new broad band appeared at around 400 nm, together with an increase in the monomeric MO band. The band at around 400 nm has been considered to be due to aggregated MO molecules bound to PLL.[13] After 800 min, the dimeric dye species at 367 nm almost disappeared. Figure 2 shows the time dependence of the CD spectra of the PLL HBr–MO complex under the same experimental conditions. Upon irradiation at 360 nm, the magnitudes of the induced CD in the 300—450 nm region decreased, and the extrema and crossover wave lengths shifted to the shorter wave length region. In agreement with the results of the absorption spectra described above, the induced CD of the PLL HBr–MO complex almost completely disappeared after 800 min. Non-filtered light (1.3×10^{20} photons cm^{-2} s^{-1}) from a mercury lamp caused much faster spectral changes than those of a filtered light and the induced CD disappeared after 10 min. Filtered ultraviolet light (360 nm) caused similar changes after 800 min and visible light (460 nm, 2.1×10^{19} photons cm^{-2} s^{-1}) after 1000 min.

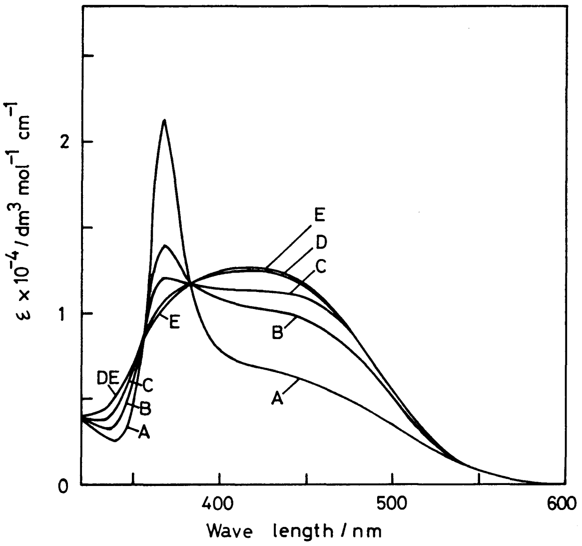

Fig. 1. Absorption spectra of the PLL HBr–MO complex in water at pH 6 and R/D=5 upon irradiation at 360 nm. A: At 0 min, B: after 200 min, C: after 400 min, D: after 600 min, E: after 800 min.

The inherent n-π^* peptide transition of an extended random coiled PLL HBr–MO complex gave an ellipticity value of $[\theta]_{217}$=2500 (based on lysyl residues) at pH 6 and R/D=5 before irradiation. The value decreased to 1000 after irradiation at 360 nm for 800 min. The decrease of the induced CD and n-π^* peptide transition was almost parallel as can be seen from Fig. 2. This observation is the same as for the CD results of water-soluble PLG with azobenzene side chains.[4,5]

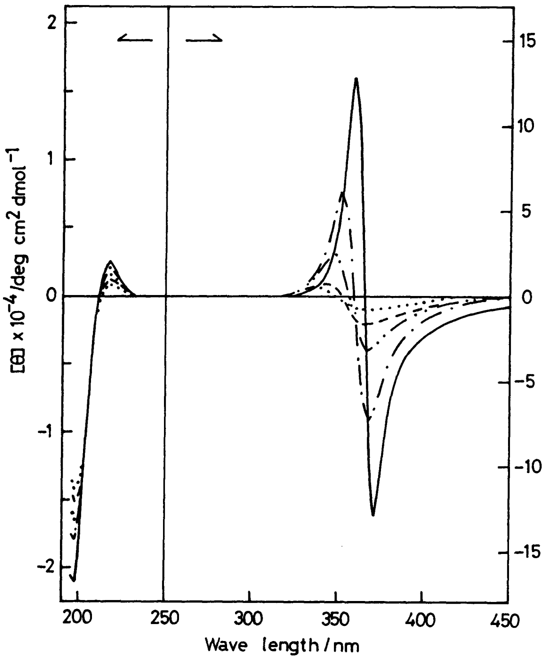

Fig. 2. CD spectra of the PLL HBr–MO complex in water at pH 6 and R/D=5 upon irradiation at 360 nm. ——: At 0 min, —·: after 200 min, —··: after 400 min, ---: after 600 min, ······: after 800 min.

MO in water does not cause an isomerization from *trans* to *cis* upon irradiation of light.[14] The reason for the spectral changes in Figs. 1 and 2 is due to the change in the interaction mode of the bound dye on the PLL, that is, from dimeric to aggregated and monomeric states. The irradiated sample (at 360 nm) left in the dark or upon re-irradiation by the visible light did not return to its original absorption and CD spectra. Thus, the change in the induced CD spectrum by light is irreversible within several days.

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